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BACTERIOLOGY
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FOURTH EDITION

Gould's Pocket Pronouncing Medical Dictionary

By GEORGE M. GOULD, A.M., M.D.

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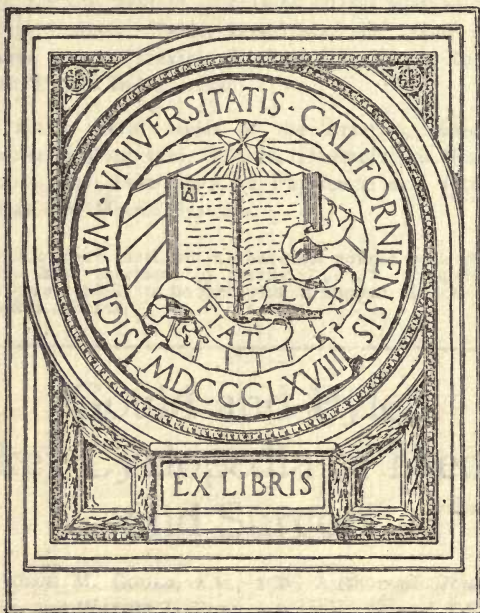
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PREFACE

This little book was designed by the writer to serve the needs of the medical student preparing for examination, and for the practitioner of medicine who desires to acquaint himself with the principle facts of the rapidly growing science of bacteriology. An effort has been made to reduce the subject matter to as concrete a form as possible.

While the literature of the subject of immunity is as vast almost as the rest of bacteriology, yet it is hoped that the chapter in this book on immunity gives in outline the essential accepted teachings on the subject.

Minute details of cultures and technic are not given. They must be sought for in books on descriptive bacteriology.

The author has drawn very freely from many standard textbooks. Many illustrations are from Kolle & Wassermann's Atlas, Park and Williams, Williams, McFarland, Tyson's Practice and Abbott.

The writer's best thanks are tendered to Dr. Herbert Fox of the University of Pennsylvania (Pepper Laboratory) to whom entire credit is due for the chapters on filterable viruses; the rearrangement of the chapters, and the new matter that has been added throughout the book.

ROBERT L. PITFIELD.

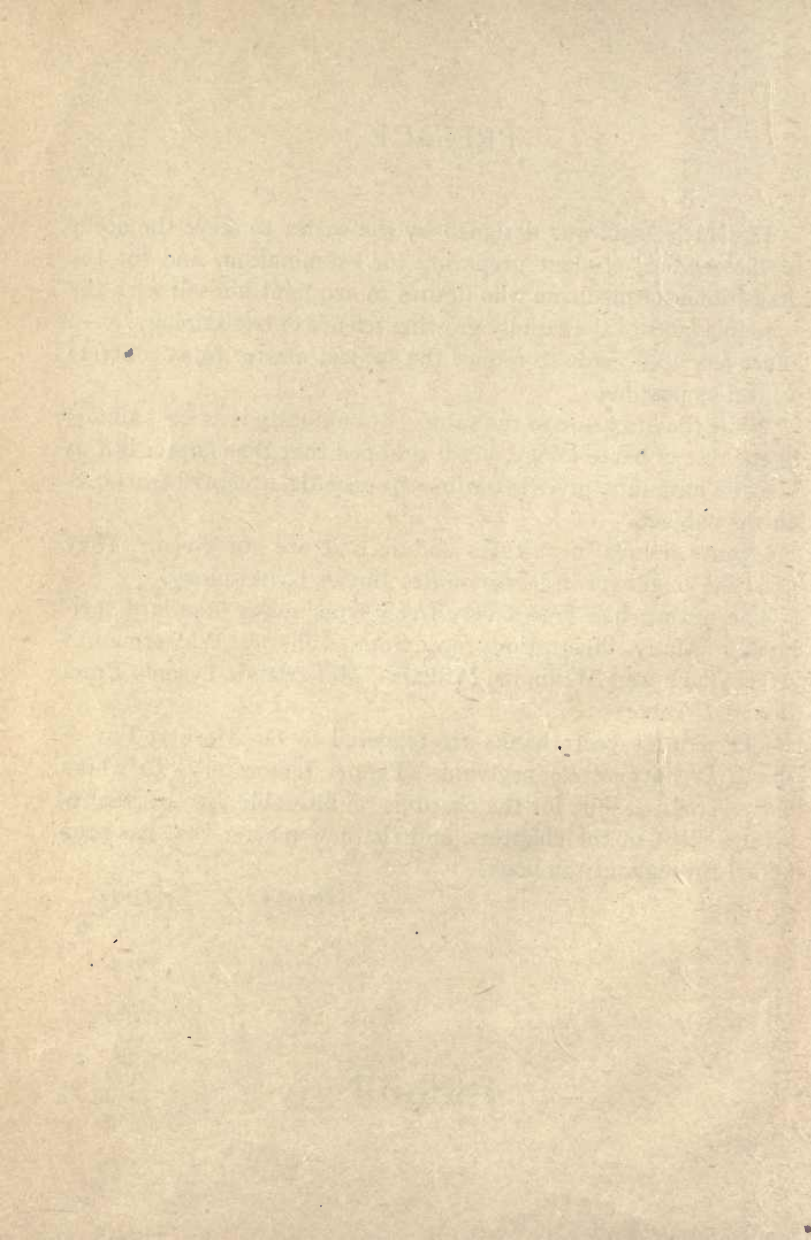


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COMPEND OF BACTERIOLOGY

CHAPTER I

THE CLASSIFICATION, MORPHOLOGY, AND THE BIOLOGY OF BACTERIA

BACTERIA (fission fungi or **schizomycetes**) may be defined as very minute unicellular vegetable organisms, almost always devoid of chlorophyll, and generally unbranched, that reproduce themselves asexually by means of direct division or fission, spores, or gonidia. They are allied closely on the one hand to the higher fungi, such as the moulds, and on the other to the algæ. Many forms in one phase of development closely resemble members of other groups, and it has always been difficult to classify them. Various botanical classifications have been employed by different bacteriologists. The following one is based somewhat upon *Migula's*, and that adopted by *Lehmann and Neumann*, which was compiled from the systems of *Flügge, Fischer, Löffler, and Migula*.

CLASSIFICATION.—Bacteria may be conveniently divided into six families, according to their morphology or shape.

- I. COCCACEÆ.**—**Spherical or spheroidal bacteria.** Globular in free state but usually seen with one axis slightly larger. They do not have parallel sides like the bacilli. To multiply, the cell divides into halves, quarters, or eighths, each of which grow again into perfect spheres. Endospores and flagella are very rare (*Lehmann and Neumann*). If mobile they are called *Planococcus* or *Planosarcina*.

- (a) **Streptococcus**.—Cells that divide in one direction only and grow in chains.
- (b) **Micrococcus**.—Cells that divide in two directions, or irregularly; with this group **staphylococcus** may be classed. Also tetrads, which form into fours by division in two directions.
- (c) **Sarcina**.—Cells that divide in three directions so that bale-like packages, or blocks of eight are formed. At least one variety (*Sarcina agilis*) is motile, having flagella. Plates of cocci, one thick in the plane, are called "*merismopedia*."

II. BACTERIACEÆ.—**ROD bacteria** are straight or slightly curved. Each cell is from two to six times as long as broad. Division takes place in one direction only, and at right angles to the long axis. Spores may be produced or may not. They may have flagella, or may not.

- (a) **Bacterium**.—Neumann—Have no endospores. Migula—no flagella.
- (b) **Bacillus**.—Neumann—Have endospores, and often grow in long threads. Migula—Flagella present at any part of cell, peritrichic in arrangement.
- (c) **Pseudomonas**.—Have endospores very rarely. Flagella only at ends.

III. SPIRILLACEÆ.—**Spiral bacteria**. Unicellular, more or less elongated. Twisted more or less like a corkscrew. Cells are sometimes united in short chains. Generally very motile. Spores are known in two varieties only.

- (a) **Spirosoma**.—Rigidly bent. No flagella.
- (b) **Vibrio** or **Microspira**.—Cells that are rigidly bent like a comma, and have always one, occasionally two polar flagella.
- (c) **Spirillum**.—Are long and spiral, like a corkscrew, are rigid, and have a bunch of polar flagella.

- (d) **Spirochæta**.—Cells with long flexible spiral threads, without flagella. Some move by means of an undulating membrane. These have been thought to belong to the bacteria but those that move by an undulating membrane should be classified with the protozoa.

IV. MYCOBACTERIACEÆ.—Cells as short or long filaments, which are often cylindrical, clavate (club-shaped), cuneate or irregular in outline, and display true or false branching. Spores are not formed, but gonidia are. They have no flagella, and division takes place at right angles to the long axis. There is no surrounding sheath as in the next family (V).

- (a) **Mycobacterium**.—Cells are short cylindrical rods, sometimes wedge-like, bent, or Y-shaped: long and filamentous. They exhibit true branching, and perhaps produce coccoid elements and gonidia, but no flagella. The **Corynebacterium** of *Lehmann and Neumann* belongs to this group. Many are *acid-fast*.

- (b) **Streptothrix** or **Actinomyces** (*ray fungus*) are long mycelial threads, that radiate in indian-club, or loop-like forms, with true branching and delicate sheaths, devoid of gonidia and flagella. Growth coherent, mould-like and dry. Often powdery on the surface in culture media, frequently emitting a musty odor. A few species are weakly acid-fast.

V. CHLAMYDOBACTERIACEÆ.—Sheathed bacteria. Cells are characterized by an enveloping sheath about branched and unbranched threads. Division takes place at right angles to the long axis of the cells.

- (a) **Cladothrix** are distinguished by false dichotomous branching. Multiplication is affected by separation of whole branches, and by swarm spores or motile gonidia having flagella.

- (b) **Crenothrix**.—Filaments are fixed to a nutrient base. Are usually thinner at the base than at the apex, formed of unbranched threads that divide in three directions of space, and produce in the end two kinds of gonidia, probably of bisexual nature.
- (c) **Phragmidiothrix**.—Cells are first united into unbranched threads by means of delicate sheaths, branching threads are then formed. Division takes place in three directions of space, producing sarcina-like groups of gonidia, which, when free, are spherical.
- (d) **Thiothrix**.—Are unbranched cells, sheathed, without flagella, divided only in one direction, and contain sulphur granules.

VI. BEGGIATOACEÆ.—Cells united to form threads that are not sheathed: have scarcely visible septa; divide in one direction, and motile only by an undulating membrane, not by flagella.

- (a) **Beggiatoa**.—Cells containing sulphur granules.

Bacteria may furthermore be classified according to their biological characteristics, which may be wonderfully different. The ultimate differentiation of one species from another depends not only on the morphology, which may be precisely similar, but on its biological behavior in culture media and in the tissues of animals under identical conditions. Again, different individuals of a given species may vary extraordinarily one from another in form and size, yet the chemical behavior is invariably the same. Hence it is only by observation of the development of bacteria in culture media, and the reactions produced in it, and in the bodies of experiment animals, that we can identify them positively from others of a foreign species. No bacteriologist is able by a simple microscopical examination of a given bacterium, to identify it absolutely at all times.

The higher groups of fungi may be classified conveniently as follows:

BLASTOMYCETES-YEASTS.—Budding fungi. Characteristic lies in predominant round or elliptical unit; some few form mycelia; division by endospores or budding; important in fermentation and in disease. Divided into:—

Saccharomyces.—Endospores and budding, fermenters. No mycelia.

Monilia.—Budding. No spores—mycelia—fermenters.

Oidia.—Budding. No spores—mycelia—non-fermenters.

Coccidioides.—Spores.—No budding—mycelia — non-fermenters.

HYPHOMYCETES-MOULDS.—Mycelium-forming fungus; division by spores, branching, budding, or intercalary division; some bisexual. Divided into:—

Phycomycetes.—Mucorinæ—sometimes bisexual—division by grouped spores or segmentation of mycelium. Not important pathologically. Example—Mucor.

Mycomycetes.—Asexual forms dividing by spores in a sac or by end organs—sexual forms dividing by specially developed cells. Mycelia predominate. Example—Aspergillus.

These are the principal groups of yeasts which can be reasonably well classified. There are others, Microsporon, Trichophyton, and Sporothrix, that have a decided pathogenic importance but for which a systematic position is not easy to give. They belong probably between the two above classes in that mycelial growth with lateral budding and spore formation are their characters.

Bacteria that are globular in form are called *cocci*.

Cocci that divide in one direction of space and grow in chains are called *streptococci* (Fig. 1).

Cocci that divide irregularly and form pairs of fours, or irregular groups, are called *micrococci*. Those of this class that form pairs are frequently called *diplococci*.¹ When they form fours by division in two directions, they are called *tetrads*. But when they

¹ This word is frequently used as if it were a biological term indicating some species identity, e.g., *Diplococcus pneumoniae*. There is no biological group called *Diplococci* and the term should be used in a descriptive sense. The cause of pneumonia is now called *Streptococcus pneumoniae*.

divide irregularly and form masses resembling bunches of grapes, they are spoken of a *staphylococci* (Fig. 2).

Cocci that divide in three directions are called *sarcinæ*. One single coccus, by division in three directions, forms cubes of eight

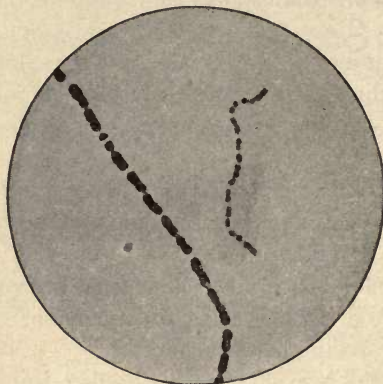


FIG. 1.—Large and very large streptococci. (Kolle and Wassermann.)

or more, each of which becomes globular and equal in size to the parent.

Motile micrococci are those that divide in two directions of space and have flagella. They are known as *planococci*.



FIG. 2.—Staphylococci. Streptococci. Diplococci. Tetrads. Sarcinæ. (Williams.)

Micrococci that divide in *three* directions, and are motile, are called *planosarcinæ* (Fig. 3).

Bacteria that resemble straight rods are called *bacilli*. These may be short and thick, or long and thread-like; are never curved, but may be slightly bent.

Bacilli may grow singly or in chains; may be flagellated; contain spores and gonidia; or, may be devoid of flagella.

Members of the spirillaceæ that resemble a curved rod, or are

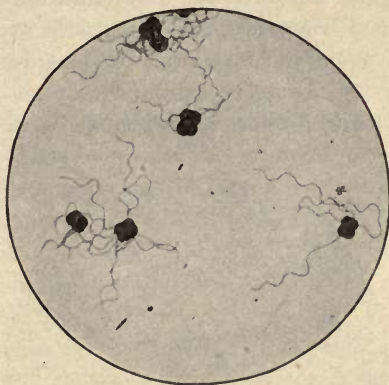


FIG. 3.—*Planosarcina ureæ*, showing very long flagella.
(Kolle and Wassermann.)

comma-shaped, are known as *vibrios* (Fig. 4). Those of the same family that resemble a corkscrew, are called *spirilla*. When they are like long spiral threads they are called *spirochætæ* (Fig. 5).

Any of these different members of the family of Spirillaceæ may grow in chains.

In clinical medicine it is common to speak of the *streptococcus pneumoniae* as the pneumococcus. As the organism appears in the diseased lung, or in the sputum, one diameter of the coccus is invariably longer than another, and the rule of equal diameters cannot be applied to it. But in culture media, the organism resembles a true coccus, being globular and growing in chains. It is then called the *Streptococcus pneumoniae*. It is common also to speak of members of the family of *Mycobacteriaceæ* as bacilli, as they are more



FIG. 4.—Cholera vibrios.
(Greene's Medical Diagnosis.)

commonly met with in this form in clinical examinations, and in cultures. Hence, we frequently hear of the bacillus of tuberculosis, and not the *Mycobacterium tuberculosis*.

Among the higher bacteria, the differentiation of those belonging to the sheathed group, or *Chlamydobacteriaceæ*, is difficult, as it depends largely upon the formation of the false branching and the gonidia. When bacteria exhibit many, or various forms, in the same culture, as does the typhoid bacillus, we speak of

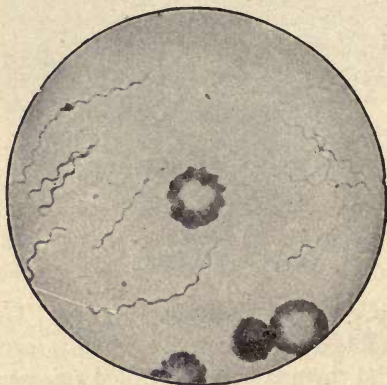


FIG. 5.—Spirochæta of relapsing fever. (Kolle and Wassermann.)

them as *pleomorphic*, or as showing pleomorphism. To elucidate: Man is pleomorphic, because among adult individuals some are tall or short, fat or thin.

Involution or Degeneration Forms.—When the best or optimum conditions for bacterial life (see page 18) are not found, bacteria present appearances quite different from those of the young, active or perfect adult type. These changes are called *involutionary* if temporary, or *degenerative* if permanent. For example: the diphtheria bacillus under good conditions for life is a straight or slightly bent rod staining in a granular manner.

If living under unsuitable conditions it becomes quite short, and stains solidly. Again, bacilli that are accustomed to appear as short elements may grow to long threads without dividing, or swell into unrecognizable form. Branching is sometimes seen in rods and spirals, a condition due in certain cases to involution, in others naturally among the higher bacteria.

To measure bacteria, we use the thousandth part of a millimeter, called the micromillimeter, or micron, as the unit. The Greek letter μ is the symbol for this unit. A micron is about $\frac{1}{25,000}$ of an inch, yet a bacterium $1\ \mu$ long, and $\frac{1}{2}\ \mu$ in width, is very large in comparison to some things that scientists measure, such as the thickness of oil films, soap bubbles, or light-wave lengths, in which the unit is a micromicron, and is symbolized by $\mu\mu$. The shortest light-wave lengths are about $400\ \mu\mu$, or $.4\ \mu$, while chromatic threads in cells of bacteria are often $100\ \mu\mu$ in width. Then again there are many things smaller than these threads. The thinnest part of a bursting soap bubble is but $7\ \mu\mu$ in thickness. There are certain infectious agents that are submicroscopic; that is, invisible even by the aid of Siedentopf's ultraviolet microscope, which shows objects smaller by half a light-wave length ($.2\ \mu\mu$).

The structure of the bacterial cell is very simple, consisting of a delicate poorly staining limiting membrane or wall enclosing a mass of substance with strong affinity for basic dyes like methylene blue. Just what part of the bacterial interior is cytoplasm and what is nucleus is not definitely known. Some observers believe that all that is stained is chromatin, or nuclear matter diffusely distributed through the bacterial cell, while others think that a delicate cytoplasm exists under the wall and that it is overshadowed by relatively great proportional bulk of the nucleus.

In the course of the rod we often see metachromatic bodies, called the *Babes-Ernst* granules, and unstained spaces called *vacuoles*, both of which are common to many bacteria. They

may be ingested substances but some are lipoidal or carbohydrate in nature. These bodies are demonstrated by staining with basic dyes and may be of importance in determining the mycobacteria. It is thought that they play a rôle in reproduction (Fig. 65).

The food of the bacterium passes through the cell wall by osmosis. The cell wall of certain organisms, for example the

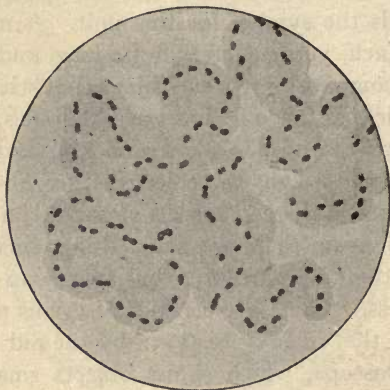


FIG. 6.—Zoöglea formation. (*Leuconostoc*.) (Kolle and Wassermann.)

pneumococcus, undergoes a change whereby a mucilaginous or gelatinous *capsule* is formed outside the cell wall. Its use is not known. The cell wall is generally the first portion of the cell to be attacked by certain specific substances (ferment) found in the blood of immunized animals, called *bacteriolysins* and *agglutinins*. Where great masses of bacteria are clumped in excessive mucilaginous material we speak of this condition as zoöglea (Fig. 6).

We sometimes find, as a prolongation of the cell wall, filamentous organs of locomotion known as flagella. Bacteria without flagella are sometimes called gymnobacteria, those possessing

them, trichobacteria but these terms are falling into disuse because the latter is now-a-days applied to higher groups that grow in hair like forms. However the following may be described: When they have one flagellum we call them *monotrichous* bacteria, and *amphitrichous* when there are two flagella, one at each pole (Fig. 7). When the cell is surrounded by flagella, it is known as a *peritrichous* bacterium, and *lophotrichous* when the flagella are arranged in tufts of two or more. These are simple adjectives and not

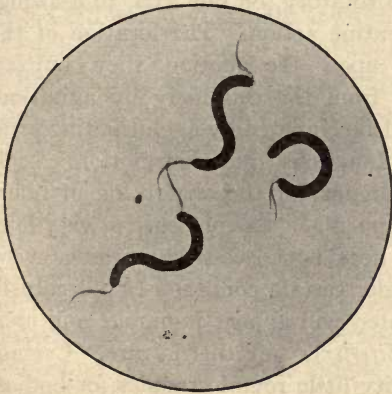


FIG. 7.—*Spirillum undula* with polar flagella. (Kolle and Wassermann.)

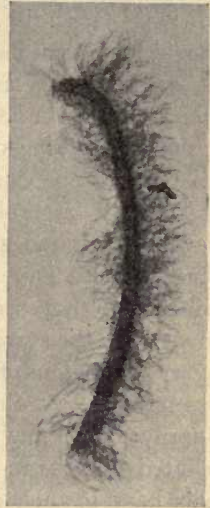


FIG. 8.—*Bacillus proteus vulgaris*, showing peritrichous flagella. (Kolle and Wassermann.)

now used as terms of classification. The tetanus bacillus is an example of a peritrichous organism, while the bacillus of green pus is called monotrichous, because of its single flagellum.

Flagella are not pseudopods, but distinct organs of locomotion.

In certain bacteria of the Beggiatoa, locomotion is accomplished by a peculiar amœboid motion, or by an undulating membrane. On looking at bacteria known to have no powers

of voluntary motion, they are seen to oscillate, tremble or move slightly. Suspensions of india-ink in water are seen to do the same thing, as are other inanimate suspensions. This molecular movement is known as the Brownian motion. By ordinary staining methods, and in preparations of living bacteria known to be flagellated, these organs of locomotion cannot be seen, as a rule. Occasionally, however, one may be seen under either condition. Generally, strong solutions of aniline dyes, to which powerful mordants have been added, are necessary to stain the capsule of bacteria and the attached flagella. The motion of bacteria varies from a simple rotatory, on one axis, to a swinging, shaking, boring or serpentine action. The location of the flagella has some influence upon the motion they impart. Flagella may be broken off from the cell body by agitation, but when separated may still be clumped by agglutinating sera.

Flagella may have other functions than locomotion. It is possible that they serve as organs for the absorption of nourishment from the surrounding media. The presence of very long or very numerous flagella does not necessarily presage very active motion. At times, under certain conditions, an organism ordinarily motile and flagellated will appear immobile and non-flagellated (*Lehmann and Ziferler*), but this is rare. Certain flagella have in their continuity little round granules, or bodies, which apparently have nothing to do with the functions of locomotion but may have something to do with the nutrition of the cell. The test of motility of a bacterium is to see it progress by itself completely across the field of the microscope.

REPRODUCTION.—The process of direct cell division is the commonest way by which bacteria multiply; hence comes the name of fission fungi. The ways of reproduction of the bacteria high in the scale are by direct division, branching, and by means of *spores*, and by other granules called *gonidia*. The spores appearing in the lower bacteria, bacilli for example, are not reproduction forms but states of high resistance.

The process of direct or binary division is very simple, and may be a matter of twenty minutes, or as long as six hours. Division is almost always across the cell in the direction of the short axis, though it may in some bacteria be in a direction parallel to the long axis, but this is uncommon.

By means of the hanging-drop or the block-culture method, on an inverted cover-glass the process may be observed easily. The phenomena of division begin by an elongation of the cell, soon followed by a constriction of pinching in of the cell on both sides, at an equatorial point. The process begins to be apparent in the cell wall and extends inward.

Division may occur in one, two, or three directions, or planes.

By cell division bacteria multiply by geometrical progression. One cell at the end of a period becomes two, and at the end of a second period these two become four; at the end of another period these four become eight; after twenty-four hours they may number many millions.

It is well that the food supply soon gives out and that the products of bacterial metabolism, such as acids and ferments, inhibit their growth. By this rapid bacterial multiplication, carcasses of animals are disintegrated and the higher nitrogenous compounds are reduced to simple gases that are quickly dissipated in the air.

SPORULATION.—Sporulation is of two kinds: the first and most important for hygiene is that into which some pathogenic bacteria go when they meet unfavorable conditions and it affords protection against all but the most vigorous disinfection; the second kind is a specialized function of the higher bacteria and moulds by which reproduction occurs (vegetative). In the latter case it is not impossible that some sexual specialization occurs. The first mentioned are called *Endospores*.

Vegetative sporulation corresponds to the flowering of the higher plants, and is observed under the most favorable vital conditions. Endospores are produced under stress of circum-

stances, when certain agencies or conditions, such as absence of food, drying, and heat, threaten the extinguishment of the organism. Spores are bright, shining, oval, or round bodies, which do



FIG. 9.—The formation of spores. (After Fischer from Frost and McCampbell.)

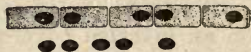
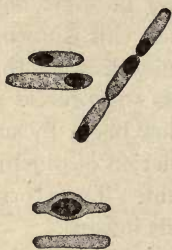


FIG. 10.—Spores and their location in bacterial cells. (After Frost and McCampbell.)

not take aniline dyes readily, and which, when they are stained, retain the color more tenaciously than the adult cells. They resist heat, often withstanding a temperature of 150°C . dry heat for an hour. Steam under pressure at a temperature of 150°C . will invariably kill them after a short exposure.

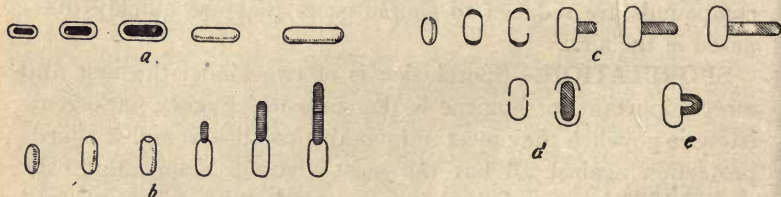


FIG. 11.—Spore germination. *a*, direct conversion of a spore into a bacillus without the shedding of a spore-wall (*B. leptosporus*); *b*, polar germination of *Bact. anthracis*; *c*, equatorial germination of *B. subtilis*; *d*, same of *B. megaterium*; *e*, same with "horse-shoe" presentation. (After Novy.)

Spores are situated either in the ends of the adult organism (polar) or in the middle (equatorial), and the spore is discharged (sporulation) either from the end or through the side.

The spore is developed in the bacterial cell as follows: If the organism is a mobile one it becomes quiet before sporulation, during which the flagella are retained. The position of the spore is early marked by a granularity of the bacterial body at one point, an area soon assuming a clear glistening character, often with a double contour, which may or may not increase the thickness of the cell. If unfavorable conditions continue the cell body disintegrates and disappears leaving the spore bare.

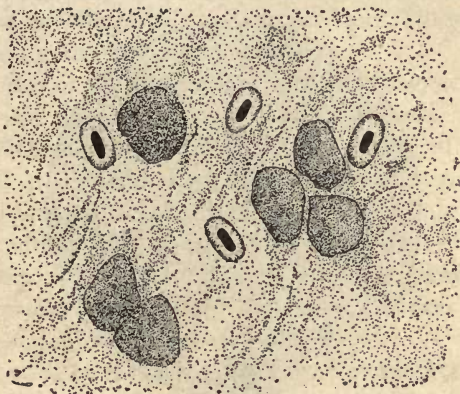


FIG. 12.—Capsules. *Bact. pneumoniae* (Friedlander). (After Weichselbaum from Frost and McCampbell.)

Certain spore bearing bacteria grown for a week at 42°C. lose the power to form spores; likewise their progeny. As a rule the anthrax bacillus does not form spores in the bodies of animals. Free oxygen is required for sporulation by some bacteria. One spore only is produced by an adult cell. Some forms of bacteria can be differentiated from each other only by the way in which they sporulate, whether from the poles or the equator.

Spores are formed chiefly by the rod-shaped bacteria especially the anaerobic and saprophytic organisms and these varieties always have a high thermal death-point. Certain round bodies

found in bacteria of high thermal death-point, are called by Heuppe *arthrospores*. It is believed that they are without significance. Arthrospores are common among the micrococci and may be associated with capsule formation and cell enlargement. The whole cell may stain more intensely. They are also to be sought among the *Streptothrix* genus.

Spores resist chemicals for a long period, and withstand drying, even in lime plaster, for years. It is believed that the thick capsule enables them to resist these deleterious agents.

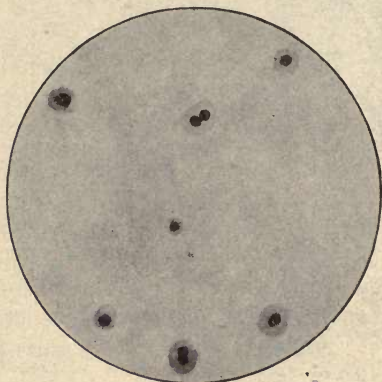


FIG. 13.—Pest bacilli showing capsules. (Kolle and Wassermann.)

Sporulation is more apt to occur under poor nutritive conditions.

The anthrax bacillus thrives at 13°C . but cannot sporulate below 18°C . Anthrax spores have been known to resist the germicidal action of a 5 percent carbolic acid solution for forty days.

Capsules.—Certain well-known pathogenic bacteria have thick well-marked capsules. The *pneumococcus*, *pneumobacillus*, and *Bacillus aerogenes capsulatus*, are well-known examples of such capsulated organisms. The capsule is not always constant. It often disappears when the organism is grown in culture media (Figs. 12 and 13).

The *higher bacteria* are those from the *Mycobacteriaceæ* up to the yeasts and moulds. They are higher than the *Bacteriaceæ* because they tend to form truly or falsely branching filaments and specialized segments, gonidia, which may behave as sex organs. Few of them are pathogenic, except in the genera *Mycobacterium* and *Streptothrix*. To the former belongs the diphtheria and tubercle bacillus, both of which are said to have branching involution forms, while to the latter belong the organisms of actinomycosis and Madura foot. The *Chlamydo-bacteriaceæ* and *Beggiatoa* are Saprophytes. These require special technique for the laboratory culture.

The *Yeasts* or *Blastomycetes* or budding fungi are next in order. They consist of sharply and doubly outlined, refractive, oval bodies which may grow out into short stalks called mycelia. They grow well in the laboratory and may produce pigments. They are much larger than the bacteria (10–25 μ long). They multiply by budding with a separation and removed growth of the young form. They may produce a local or general infection in man, Blastomycosis. They are used in beer making. The commonest genus is *Saccharomyces*.

The *Moulds* or *Hyphomycetes* represent the next highest group of the plant algæ. They are characterized by a greater prominence of the mycelium over simple segments or bodies. They are widespread in nature and many are pathogenic. They multiply by segmentation of the mycelia into gonidia or by the development of special spore masses called sporangia. Further refinements of the spores into sexual elements is known. They are chiefly of interest to the physician on account of the skin diseases that they occasion.

THE CHEMICAL COMPOSITION OF BACTERIA

Bodies of bacteria contain water, salts, certain albumins, and bodies that may be extracted with ether. Among the latter are lecithin, cholesterin, and triolein. In acid-fast organisms, fatty

acids and wax have been found. In others, xanthin bases, cellulose, starch, chitin, iron salts, and sulphur grains have been discovered. The essential protein of the cell body is highly nitrogenous and is usually combined with some carbohydrate as a glyconucleo-protein. The salts in the ash are mostly composed of various phosphates. Intracellular toxins in combination with the cytoplasm are found in certain groups of bacteria, e.g., *B. typhosus*.

BIOLOGICAL CONDITIONS

Bacteria are arbitrarily classes as *parasites*, or *saprophytes*. They may be so dependent upon the tissues of the infected organism as to be a strict parasite and incapable of growth under any other condition (*Mycobact. lepræ*), or they may be capable of life on artificial culture media (tubercle bacillus), or of life in the body, on culture media containing organic matter (influenza bacillus), or in the soil (*B. tetani*).

Saprophytes are bacteria capable of living upon dead organic matter, in soil, in water, in air; they are not parasitic and do not resist the defenses of the living body.

Certain biological conditions are essential for the growth of bacteria: water, oxygen, carbon, nitrogen, and salts are necessary. For certain parasitic bacteria, highly complex substances are indispensable: meat albumins, peptones, milk, egg albumin, blood serum, and sugars are the ingredients of various culture media.

The chemical reaction of such media is important: it should either be faintly acid or faintly alkaline. The greatest number of water bacteria grow in media that are slightly acid, while diphtheria produces its strongest toxins and grows best in alkaline media. Salt-free media is required for a number of pathogenic bacteria, e.g., the gonococcus, *B. lepræ*.

All bacteria require for their growth either free oxygen, as in air, or combined oxygen, as in albumin, water, etc. Those that

only grow when deprived of free oxygen are known as *obligate anaerobes*, while those that require the presence of oxygen are called *obligate aerobes*. Those that grow under either conditions are named *facultative anaerobes*. Free oxygen is needed for spore formation by certain bacteria. Anaerobes obtain oxygen as they need it by breaking up their foodstuffs.

Nutriments are most important for the growth of bacteria, nitrogenous compounds (albumins) particularly being required. Simple aquatic forms of bacteria can live and grow in distilled water. The addition of the various sugars is of advantage in the cultivation of many bacteria, and glycerine for the growth of some members of the *Mycobacteriaceæ*. Blood serum or whole blood is required by some pathogenic organisms. The foodstuffs must be in a form that can diffuse through the cell wall.

The **temperature** of the medium in which various bacteria grow is most important. Bacterial growth is possible between 0°C . and 70°C ., some varieties thrive at the one extreme, and others at the other.

Psychrophilic bacteria, are those that grow at 15°C ., with a maximum of 30°C . and a minimum of 0°C . Water bacteria of the polar seas belong to this group.

Mesophilic grow best at 37°C .—the temperature of the body—and thrive from 10°C . (minimum) to 45°C . (maximum). All pathogenic bacteria belong to this group.

Thermophilic (min. temp. 40°C ., max. 60 – 70°C .) are most prolific at 50 – 55°C . To this class belong bacteria of the soil. All of this class are spore-bearing.

Darkness favors bacterial growth.

Association of different kinds of bacteria is of some importance in their growth and welfare and when thus associated, they sometimes benefit each other. Such combination is called *symbiosis*. Antibiosis is the condition when one or more of a mixture of organisms suffers by the presence of others, *e.g.*, the destruction of putrefactive germs in the intestinal tract by lactic acid bacilli.

Certain anaerobic bacteria grow in the presence of oxygen if other particular varieties of aerobic bacteria are present.

Attenuated tetanus bacilli become virulent if cultivated with *Bacterium vulgae*. Again, complicated chemical changes, as the decomposition of nitrites with the evolution of nitrogen cannot be accomplished by certain bacteria severally, but jointly, this is quickly brought about.

Pfeiffer has shown that certain chemical substances (foods, albumins, etc.), attract bacteria (*positive chemotaxis*), while other substances, as turpentine, repel them (*negative chemotaxis*). Oxygen repels anaerobes and is particularly attractive to aerobes.

FREE AGENTS PREJUDICIAL TO THE LIFE OF BACTERIA

High temperatures are surely germicidal: 60°C . coagulates mycoprotein of bacteria and other common albumins. The degree of temperature at which bacteria are killed is called the *thermal death-point*. Most vegetative forms die after a short exposure at 60°C ., though some require a higher temperature, *e.g.*, tubercle bacillus.

Spores resist boiling, often for hours. Spore-bearing bacilli from the soil often survive a temperature of 115°C . moist heat (steam), from thirty to sixty minutes. Bacteria resist dry heat of 175°C . from five to ten minutes.

Cold inhibits bacteria; destroys some; but is not a safe germicidal agent, as typhoid bacilli have been isolated from melted ice in which they had been frozen for months.

Ravenel exposed bacteria to the extreme cold of liquid air (-312°F .) and found that typhoid bacilli survived an exposure of sixty minutes; diphtheria, thirty minutes, and anthrax spores, three hours; during this exposure, however, many were destroyed.

Light is inimical to the life of bacteria, direct sunlight being the most germicidal, as it destroys some, reduces the virulence of

others, or interferes with the chromogenic properties. Typhoid, cholera, diphtheria, and many other organisms are killed after an hour or two's exposure to bright sunlight. The ultraviolet or actinic rays are the efficient ones. If free oxygen is excluded, the germicidal action is very materially reduced. Sunlight acting on culture media (free oxygen and water being present) produces after ten minutes, peroxide of hydrogen. This action of light on bacteria has been extensively used, notably by Hansen, as a therapeutic measure for the cure of bacterial skin diseases, especially lupus. Diffuse sunlight, electric light, Röntgen-rays, continuous and alternating currents of electricity, are also more or less germicidal. Antiseptics, such as metallic salts, formalin, carbolic acid, cresol, mineral acids, and essential oils, are powerful germicides; some even in high dilution.

According to Koch, absolute alcohol, glycerine, distilled water, and concentrated sodium chloride solution do not affect anthrax spores, even after acting on them for months. Halogen elements (iodine, bromine, chlorine) are the most powerful germicides.

Free acids and alkalis must be very strong to act as disinfectants. Excessive amounts of sugar, salt, glycerine, and the pyroligneous acids act as destroyers, or inhibitors to bacterial growth in foodstuffs.

Metals act as lethal agents in the presence of light and water, by forming metallic peroxides, which either destroy the vitality of bacteria or hinder their growth. Silver, zinc, cadmium, bismuth, and copper, have this action. Consequently silver wire and foil, are used in surgery because of their antiseptic action. Metallic fillings in teeth prevent the growth of bacteria that cause caries.

Certain *cells* in the *bodies* of animals (leucocytes) and some elements of the blood serum, being bactericidal, are a powerful means of internal defense against infection.

If the water of the cytoplasm of bacterial cells is dried out, the vitality of the organism suffers. The length of time required for drying varies, anthrax spores resisting the process for over ten

years. Ancient methods of preserving foods from putrefying, and which are still in vogue, depend upon the employment of some of these agents, which are prejudicial to bacterial life. Meats are salted, pickled, dried, or smoked. Fruits are dried, pickled, or immersed in strong saccharine solution, in order to preserve them from decay, in every instance, the absence of moisture, the excess of salt, sugar, or vinegar, or the pyroligneous acid from the smoking, prevents bacterial growth, and consequently, decay of the foodstuff. The *products* of bacterial growths often inhibit, or destroy, the cells that made them, as well as other bacteria. *B. pyocyaneus* and *S. cholerae*, have this property of secreting autolytic ferments.

CHAPTER II

PRODUCTS OF BACTERIAL ENERGY

According to their chemical activities, bacteria are arbitrarily divided into the following classes:

Photogens

Chromogens

Zymogens

Saprogens

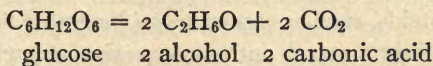
Aerogens

Pathogens

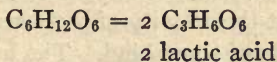
Photogens are those bacteria of the sea, putrefying flesh, and damp rotten wood, that produce a faint phosphorescence.

Chromogens are bacteria that produce colors as they grow, notable among which may be mentioned the *Staphylococcus aureus*, that are golden in hue; *B. pyocyaneus*, of a greenish-blue; and *B. prodigiosus* which appears a brilliant red.

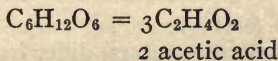
Zymogens are the bacteria of fermentation, which is the chemical transformation of carbohydrates by the action of bacteria, with the evolution of CO_2 CO & H . Such bacteria are useful in the industries for the production of alcoholic beverages, wine, beer, etc. Through the actions of these organisms grape sugar is converted into alcohol, lactic acid, and acetic acid.



or



or



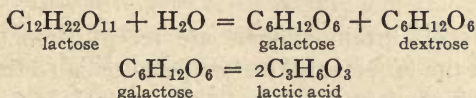
From the bodies of ground yeast cells a soluble ferment, *Zymase*, has been expressed, which causes alcoholic fermentation of cane and grape sugars. This fact proves that fermentation is not necessarily a vital process. The fermentations of bacterial enzymes may give acids, and also aldehydes, ketones, CO_2 , CO , H , N , NH_3 , marsh gas and H_2S . The carbohydrate splitting powers are used in determinative bacteriology.

Fermentation and putrefaction are bacterial enzymic processes of indispensable importance to life. Bacteria reduce excrementitious matters to their elements and then others build up these elements into conditions favorable for plants. This process affects the cycle of utility of carbon, sulphur and particularly nitrogen in the air and soil. Some soil bacteria can fix nitrogen from the air for the use of plants. Because of the importance of these processes, culture of appropriate bacteria may be spread upon exhausted soil. These are chiefly nitrifying bacteria. Manure contains the denitrifying organisms. Bacterial fermentations produce the flavor of tobacco, opium and butter.

Enzyme Production by Bacteria.—These products are difficult to define because few have been obtained in an entirely pure state. They may be described as soluble, but non-dialyzable products, precipitable by salts of heavy metals or by alcohol, destroyed by 70° but resisting drying and decomposition. They are restrained by excess of alkali, of acid, and by an accumulation of their own products. Ferments of great variety and power are formed by the zymogens, as *proteolytic*, which dissolve proteids, such as casein; *tryptic*, gelatine liquefying; *diastase*, which converts starch into sugar; *invertase*, which changes cane sugar into grape sugar; ferments that curdle the casein of milk; and it may well be that the activity of pathogenic bacteria in the body is due to ferments of some kind. The hemolytic action of the golden staphylococcus or the tetanus bacillus is thought, by some, to be of enzymic nature.

Organized ferments (bacteria, yeasts) differ from the *unorganized*

(pepsin, diastase). The latter "exercise solely a hydrolytic action" (Fischer), causing the molecules of insoluble compounds to take up water and to separate into less complex molecules of a different constitution, which are soluble in water. The organized ones act differently. Highly complex molecules are split up, and numerous substances of a totally different character are formed with the evolution of gases and by-products (Fischer). The reason for this is, perhaps, to be found in the supposition that the bacteria abstract oxygen for their own use, and thus cause the atoms to unite into an entirely different substance. According to the above-named investigator, it is not possible to express such chemical changes by a simple equation. Experiments have shown that *B. typhosus* and *pyocyaneus* are able to split up olive oil or fat, and produce glycerine and fatty acids, thus making them accessible to fermentation (Fischer). The action of the buttermilk organisms, while usually very complex, may be represented by the following:



Saprogens produce putrefaction which is the chemical transformation of albuminous bodies with the evolution of nitrogen, and of alkaloidal substances, known as **ptomaines**. Aromatic elements are also produced, such as indol, phenol, kresol, etc.

It is therefore obvious that fermentation and putrefaction are separate processes, the former an action upon carbohydrates, the latter a splitting up of proteins. It has been found that when organisms can attack both substances, the sugars and starches are first broken up; this is what is meant when it is stated that carbohydrates have a "sparing action" upon proteins.

Pathogens.—If the tissues are receptive to bacteria, and if the latter, in any way, injure the tissues, then the invading organism is called *pathogenic*. Theoretically the tissues of the body are

sterile, but as a matter of fact, isolated pathogenic bacteria such as colon and diphtheria bacilli, streptococci, and pneumococci, have been found in the tissues and cavities of the body in the absence of pathological evidence of their presence.

Sixteen hours after death the blood and tissues teem with bacteria that have wandered in from the intestines. It has been shown that bacteria, even non-motile ones, can migrate through the body during the agonal period.

Bacteria may cause disease in the following ways: (a) mechanically, a clump of bacteria may plug a capillary; (b) simply overwhelm the tissues and absorb the oxygen (anthrax); (c) they may cause new growths (tubercle); or false membranes to form in the larynx causing suffocation (diphtheria); (d) ulceration of heart valves causing cardiac insufficiency; (e) thrombosis in the veins and arteries; (f) pus formation; (g) by generating toxins that cause anæmias, or degeneration of important elements of the nervous system, parenchymatous organs and the walls of the blood-vessels.

The tissues of certain animals are receptive for particular bacteria, and the latter are therefore pathogenic to that animal. B. of swine plague is pathogenic to swine, but not to man. *B. typhosus* is pathogenic for man, but not to swine.

As emphasized above, the activities of bacteria are due to the enzymes they produce. In the course of their life, bodies, called toxins, are formed that have the power of producing illness in higher plants and animals. These bodies are similar to the enzymes. Both are produced in minute quantities. Their exact chemistry is not known, and pure toxins, at least, have probably never been isolated. We test for them by animal experiments while the presence of enzymes may be observed upon artificial culture media. Toxins of bacteria are not the only ones formed. Castor bean produces a body classed among the toxins as does the rattlesnake in its venom. These bodies differ from ptomaines, also poisons, by being less resistant to heat, in causing a peculiar

blood reaction and by refusing isolation. The toxins are not essential to the life of pathogenic bacteria and some of the usually virulent organisms may grow without toxin development. Toxin productions may be lost and regained. The real object of the toxins is not known, as it is not thought that bacteria gain anything by producing disease. They are separate from the other chemical bacterial products. Toxins may be divided into those which are secreted through the bacterial cell wall and diffuse through the median in which organisms are growing, the *extracellular* or *soluble* toxins, and those which remain within the bacterial cells and are only liberated upon their death and disintegration, the *endotoxins*. Closely related to the second class are the so-called *toxic bacterial proteins* or *plasmins*. These do not separate from the structures since bacteria which produce them furnish a toxic mass if thoroughly washed, ground and rewashed.

Examples and Characters. Soluble or Extracellular Toxins.—The best examples are those of the tetanus and diphtheria bacilli. In diseases caused by these germs, bacteria do not enter the body fluids but the general manifestations are due to absorbed soluble poisons. Such toxins are soluble in water; they are rendered inert by heating, sunlight and some chemicals. They dialyze very slowly and are not crystallizable. They may be precipitated with the albumen fraction of the medium. They may be precipitated and dried, in which state they keep much longer than when in solution, and then are more resistant to heat. Curiously enough the toxins may be destroyed by proteolytic enzymes. Some toxins are complex; the tetanus toxin for example, contains two elements, one a dissolving power on red blood cells, the other a stimulator of the motor system. They are specific for each organism.

Endotoxins.—These are exemplified by the poisons of the typhoid and plague organisms. We know little of their chemistry but we may assume that it is of protein material and similar to that of the bacterial cell. These toxins are less rigidly specific

than the extracellular poisons. They are probably quite complex in activity as they give rise to various anti-poisons when in the animal body. These poisons are resistant to heating at 80°C . and keep under artificial conditions much longer than soluble toxins.

The toxic bacterial proteins are best exemplified by tuberculin. This is complex mixture of the proximal principles of the tubercle bacillus and is probably albuminose in character. These substances are almost as specific for their own germs as the toxins and much more so than the endotoxins. They are capable of producing a reaction in animals similar to that which might be produced by the organisms themselves. For example tuberculin, wholly free from tubercle bacilli, will produce a reddening of the skin or a rise of temperature if injected into a tuberculous individual. The dead tubercle bacillary mass if placed beneath the skin of a healthy guinea pig will set up a local limited miliary tubercle. The reactions from mallein andluetin (*q.v.*) injection are due to toxic proteins. The proteins are usually thermostable, that is not destroyed at 100°C .; this is also called coctostabile.

In practice it may not be so simple to separate bacteria that produce the various poisonous elements as the above descriptions would indicate. Toxins are all in a sense specific, that is they are for the most part selective in action, and are harmless if swallowed. The diphtheria toxin is absorbed from a raw inflamed surface under cover of an exudate composed of fibrin and bacteria. The tetanus toxin is absorbed from its seal of manufacture in the depths of a punctured wound. The endotoxin of typhoid bacilli has no pathogenic effect if swallowed or rubbed in skin or mucous membrane. If it be injected under the skin in the absence of bacteria it will call forth reactions on the part of the body similar to those expressed when living typhoid germs are circulating. Toxins are again relative in their affinities. Tetanus toxin is fatal for man and horses while rats and birds are resistant to it. We use this expression of specificity for determining the nature of certain germs. We may speak of failures to react as failures

of receptivity on the part both of the microbe and the injected animal.

Other characters of toxins are that they act in dilute suspensions, are destroyed by heat, and produce, when injected in small doses into animals, a specific anti-substance.

CHAPTER III

INFECTION

Infection means the successful invasion of the tissues of the body by either animal (protozoa, vermes) or vegetable (bacteria and moulds) organisms with the evidences of their action. To successfully infect the body, bacteria must enter the tissues, be of sufficient number, find the tissues receptive, and continue to multiply.

The skin, mucous membranes, and the various cavities of the body connected with the outside air, teem with countless bacteria at all times, many of which are pathogenic, yet there is no infection, because the tissues are not invaded. Again, there can be no doubt that highly pathogenic bacteria enter the tissues of healthy people at times, in small numbers, and yet no disease is produced, because of their scarcity, or by reason of the tissues not being receptive. Infection implies not only invasion of the body, but injury to the tissue. Certain bacteria may invade a body, and yet create no harm. These bacteria may enter dead or dying body tissues, and secrete poisonous substances (toxins) which may be absorbed, and produce pathologic symptoms known as *Sapraemia*. Clots of blood in the parturient uterus, and gangrenous limbs may be invaded by strict saprophytes incapable of life in living tissues, and yet cause much harm by the absorption of their products.

Infestation is when organisms, even pathogenic, are present in a place without exciting a reaction; the term is best used however to imply the presence and action of animal parasites. Matter carrying pathogenic germs is called *infective*.

Depending upon the ability to grow in the body, bacteria may be divided into: (1) purely saprophytic ; (2) occasionally parasitic; and (3) purely parasitic. A *host* harbors a parasite.

Purely saprophytic germs cannot live in tissues at all; those that are occasionally parasitic lead a saprophytic existence in the soil or water, and yet may invade the body, and produce disease: the tetanus and malignant œdema bacilli are examples of this group. Those bacteria that are purely parasitic are only known as they exist in the tissues of the infected host, and have no outside existence at all.

Koch's Postulates

In order to prove that a certain organism is the infectious agent of a given disease, Koch has devised four postulates which the given organism must fulfill before it can be considered the cause of the disease.

1. The organism must be found microscopically in the tissues of the animal having the disease, and its position in the lesion should explain the latter.

2. It must be isolated in pure state from bodies of the diseased animals.

3. And then it must be grown for successive generations in culture media.

4. If injected into a healthy animal, or animals, it must produce the same disease, and be found in the lesions of the disease in the animal's tissues.

Some of the many organisms that certainly fulfill these conditions, are as follows:

Streptococcus Pyogenes (Sepsis). *Actinomyces*.

B. of Tuberculosis.

B. of Diphtheria.

B. of Anthrax.

B. of Tetanus.

B. of Glanders.

B. of Malignant Œdema

B. of Bubonic Plague.

B. of Malta Fever.

B. of Typhoid.

Spirillum Cholerae.

Pneumococcus (Pneumonia).

B. of Dysentery.

Meningococcus.

Spirochæta of Relapsing Fever and of Syphilis

There are several other organisms that are considered to be the cause of specific disease, but they do not fulfill the postulates. Among these are:

The Protozoa of Malarial Fever

Amæba Dysenteriae.

While the specifications outlined by Koch as indicating the etiological rôle of an organism were sufficient for the period at which they were laid down, advances in immunology have added so much information about antigens and antibodies that it is but right today to expect that a virus should behave as an antigen by calling forth certain immunity reaction under spontaneous and experimental conditions. Such an expectation is fulfilled in practically all cases, and indeed has been, even in a few instances where all Koch's postulates could not be completed, typhoid fever being a notable example.

In rheumatic fever, measles, mumps, yellow fever, chicken-pox, rabies, and dengue, the specific cause has, thus far, eluded discovery. In the case of measles, hog cholera, and some of the eruptive diseases, it has been found that the cause of these diseases resides in the blood, and if the serum of the latter is carefully filtered through a Berkefeld filter, it is still capable of producing the disease in susceptible animals. Careful microscopic search fails to show any bodies in the serum that might be considered the agents of infection, and it is thought that these organisms are submicroscopic (see chapter on Filterable Viruses).

If the invading organism is a pure saprophyte the various forces for internal defense immediately act upon and destroy it.

Bacteria are disposed of in diverse ways. By means of the

lymph channels they are carried to the various mucous surfaces of the body, intestinal and bronchial. The liver, according to Adami, destroys at once bacteria absorbed from the intestines. During typhoid fever, the typhoid bacilli are often found in the urine, the organisms escaping from the blood or from the lymphoid foci in the kidney. Pathogenic bacteria are discharged from the body in feces, pus, sputum, and in scales in the desquamating skin diseases.

To successfully inoculate a guinea pig with tuberculosis, the tubercle bacilli should be injected beneath the skin.

It has been said that successful invasion demands a sufficient number of organism; it is equally true that the number admitted will determine the character of disease to arise, as is indicated by the following observation of Cheyne.

In experimenting with the staphylococcus aureus, it was found that 250,000,000 were required to cause an abscess; and 1,000,000,000 were needed to cause death. The internal powers of defense were able to cope with or limit the action of a few million to a certain locality, but could not withstand the injection of overwhelming numbers, which caused the animal's death.

There are three attributes which make successful the invasion of pathogenic germs into the body: virulence, toxicity and pathogenicity. These factors shade into each other sometimes very confusingly and are of course capable of varying proportions in the same organism. In order to combat successfully the primary defenses of the body, a virus requires virulence, the degree and permanency with this is accomplished being due to the amount of poison the invader can elaborate to keep the safety devices of the economy from conquering. The physical damage done is attributable to the pathogenicity of the germ. It is like a fight where the man has strength, and staying powers and does physical damage to his adversary.

Ehrlich's explanation of virulence assumes that bacteria have binding posts or receptors and the more of these a germ has, the

more of the natural defenses it can anchor and remove from the field.

Their virulence can be *lessened* by cultivation at a higher temperature than the body, 42.5° – 47° C.; by drying; the exposure to light; the action of chemicals; compressed oxygen; and by passing the organism through the bodies of non-susceptible animals. The attenuation or weakening of the pathogenic powers of bacteria is useful for the production of various vaccines which are valuable in preventive medicine.

By growing the anthrax bacillus at a high temperature, 42.5° C., it becomes so avirulent that it is incapable of destroying sheep or rabbits. It is then used as a vaccine to prevent infection with virulent bacilli. By exposing the spinal cords of animals dead from hydrophobia to the action of drying for various periods, Pasteur was able to attenuate the virus, so that it would not produce hydrophobia, but on the contrary, it, by repeated inoculation, caused immunity. The inoculation of monkeys (which are non-susceptible) with hydrophobia virus attenuates it. The growth of the small-pox organism in the cow, causing cow-pox, so reduces the virulence of the germ that it is incapable of producing small-pox in man, but only vaccinia; infection with this gives immunity against small-pox. The flesh of animals that have died from *quarter-evil* is so changed by heat and desiccation that if it is injected into susceptible animals, they do not succumb but are vaccinated against infection with the virulent organism.

When we speak of attenuation of virulence we usually refer to the effects on experimental animals and specify what attenuation is meant when they are to be used as vaccine. A very interesting pathogenic, yet attenuated, form of streptococcus is to be met in subacute endocarditis. These organisms produce serious or even fatal valvulitis, and yet have no effect upon lower animals. They are extremely hard to remove from the body. They have accustomed themselves to residence in the body, have estab-

lished a balance of poise between their offenses and the bodily defenses and practically cannot be rapidly dislodged. These are called *fixed* or *fast* strains. Such strains may be seen under other conditions such as the typhoid bacillus in the gall-bladder. These fast strains usually are found at places remote from intimate contact with the defenses of the body, the leucocytes and blood serum as in the cases cited.

The malignancy of bacteria may be *heightened* in various ways: (1) By passing them repeatedly through the bodies of susceptible animals; (2) by cultivation in culture media in collodion sacs placed in the abdominal cavities of animals; (3) by injections mixed with other injurious substances, such as lactic acid, and the metabolic products of foreign bacteria. Cultures of pneumococci may be made so virulent by the first means that only one pneumococcus is capable of setting up a fatal septicæmia in a rabbit. By injecting attenuated diphtheria bacilli with streptococci into a rabbit, the virulence of the bacilli can be raised, as mixed infection often adds to the virulence of an organism. Malignant streptococcic infection added to virulent diphtheria infection, greatly increases the severity of the disease. The transference of infective agents from one person to another during an epidemic increases the virulent action of the organism by reason of the rapid passage from individual to individual.

Mixed infections are those in which more than one kind of virus is active. It is of course possible that two kinds may originate a disease, but it is usual for one germ to initiate a process and another to be superimposed upon it, usually intensifying the lesions. The active ulcerative inflammation in tuberculous lungs is usually due to be secondary effect of streptococci.

The secondary streptococcic infection in small-pox and in phthisis complicates the primary infection and frequently causes death of the individual affected.

The avenue of infection and the tissues infected alter the type of the disease exceedingly. Streptococci invading the tonsils cause

tonsillitis, but the same organisms entering the skin cause erysipelas of phlegmons; or if the uterus is infected after the birth of a child the disease is still different and more serious. If the tubercle bacilli enter the skin they produce lupus; if swallowed they cause ulceration of the bowels, and subsequently invade the peritoneum; if inhaled, tuberculosis of the air passages, phthisis, or tuberculous laryngitis may follow. If cholera spirilla be injected into a vein of a guinea pig, it may develop choleraic septicæmia; if they are injected into the peritoneal cavity, a choleraic inflammation of the peritoneum is produced, and not a septicæmia. Pneumococci if injected into a vein cause a rapid septicæmia, or they may give rise to abscesses anywhere in the body. Like streptococci, they may be the cause of inflammation in any tissue, particularly serous membranes, and show different clinical entities, according to the organs involved, and the morbid anatomy and physiology produced. The fatality of a bacterial infection varies with the avenue of inoculation: it is safer to have a skin infection than a meningeal, or endocardial one, not only from the likelihood of rapid toxin absorption, but from purely mechanical damage, as pressure and interference with vital functions by inflammatory products such as fibrin, tubercles, serum and pus.

How Bacteria Are Brought to the Body.—Air-borne infection may occur by the direct transference of the bare organisms, a very rare occurrence, or by dust or by droplets of fluid usually sputum. Organisms settle on objects of our environment when leaving the sick and can be stirred up with the dust. This is important for diphtheria, the acute exanthemata and tuberculosis although the most dangerous source for the last is the coughing consumptive. The transmission of pertussis and pneumonia is almost surely always a droplet convection.

Water-borne infection, including typhoid, cholera and dysentery occurs by the contamination of water courses with the discharges of the respective diseases.

Milk-borne diseases, tuberculosis, diphtheria, epidemic sore

throat and some others, occur because persons or animals suffering with the disease have handled or supplied the milk. This direct contamination also applies to food like meat and oysters (typhoid and meat poisoning).

Soil-borne diseases are chiefly those arising by direct implantation of earth into the body. Of course the ground may be soiled by discharges from infectious diseases and contamination of hands and clothing.

Animal carriers of disease include those acting as intermediate hosts (anopheles mosquito in malaria); as mechanical conveyances of a direct or indirect nature, in the former case like transmission of organisms from a sick man or animal to a well one, in the latter case transferring the germs to food consumed by a healthy being; or acting as a passive host for the germ as is the case in the transmission of plague bacilli by the rat flea.

Human transmission of infective matter is the most important of all methods as it is an axiom that a person suffering with a disease is most capable of transmitting it. This occurs by direct contact, by the carrier state and by passing the contagium to the embryo.

Carriers.—After recovery from certain diseases, notably typhoid fever, diphtheria and cholera, convalescents may carry in themselves fully virulent germs with no outward evidences thereof. Such persons are called "*carriers*" and are of the highest importance in hygiene. The reasons for this condition are several. These germs may be removed from the bodily defenses or the body may be immune to them; again they may be fixed or fast strains. Wherever they are they may escape and infect another person. After typhoid fever bacilli remain in the gall-passages and bladder; after cholera in the deep mucous membranes and after diphtheria the crypts of the tonsils or the nasopharynx may hold them. Vaccination or operation may be needed to remove them. Persons never known to have had enteric fever have been known to harbor bacilli in their gall-bladder. One typhoid

carrier, "Typhoid Mary" a cook, is known to have infected 26 persons. Such persons because of their apparent innocence might be called "hidden carriers." They have been found transmitting dysentery and poliomyelitis as well as the above typhoid fever, and, judging from the continued existence of the exanthemata in cities, it may be that we shall find such persons harboring the virus of varicella, mumps and pertussis.

Local Immunity to Infection.—There is evidently more resistance offered by the liver against invasion than by the peritoneum. It is not likely that a man would contract typhoid through skin infection, nor is it probable that he would contract tetanus by swallowing tetanus bacilli, but the reverse of these conditions certainly produces infection.

Infection may be caused from without the body, or from within. Lockjaw, sepsis, hydrophobia, or anthrax may follow injuries from rusty nails, splinters, weapons, unsterile fingers, or instruments. Personal intercourse, bites, kisses, sexual intercourse, association with persons suffering from exanthematous or contagious diseases may transmit disease.

Winslow has found colon bacilli upon 9 percent of the hands he examined. Tubercle bacilli have been found on the hands of the non-tuberculous. Some organisms, notably the smegma bacillus, pyocyaneus bacilli and cocci resembling the white pus former, may be said to be normal inhabitants of the skin.

The bites of insects that are intermediate hosts of infectious agents (plague bacilli, malarial organisms, etc.) are sources of infection from without, as is also the ingestion of infected food or water.

Infection from within may be caused by the migration of bacteria from the skin inwards, or from any of the mucous membranes, on which, and in which many pathogenic bacteria at all times may be found.

Bacteria from the mouth, stomach intestines and the rectum may invade the tissues and the blood under certain conditions.

This is particularly the case during the last stages of diseases, not necessarily infectious, such as chronic heart disease, kidney disease, or diabetes. Vital resistance is much lowered, and intestinal bacteria, invading the tissues in enormous numbers, set up what is known as *terminal infection*, which is often the immediate cause of death.

The stomach with its gastric juice, containing during digestion .2 percent to .3 percent of hydrochloric acid, guards the lower alimentary tract against infection. A great many bacteria are ingested with foods, particularly with milk, cheese, and overripe fruit. These in the most part are quickly destroyed by the hydrochloric acid. When the stomach is diseased and the contents become stagnant, as in stenosis of the pylorus, and in carcinoma, when HCl is diminished, or absent, fermentative bacteria give rise to great amount of gas, and lactic acid, to the great discomfort of the patient. The normal acidity of the stomach is a great safeguard against infection with cholera. If tubercle bacilli are swallowed, and if infection occurs, the lesion is not always localized to the alimentary tract. Lesions of the lymph glands, peritoneum, bones, and nervous tissues often follow the ingestion of these organisms. Dogs fed on soup containing great numbers of tubercle bacilli, and then killed three hours after, were found to have bacilli in the thoracic duct. Chyle from the duct, injected into guinea pigs, caused tuberculosis in them (Nicolas and Descos).

The interior of the uterus, the bladder, urine, deep urethra, and lungs are generally sterile in health. With the exceptions noted where germs are not usually found, all tissues, especially the inlets and outlets of the body, may be said to have a normal bacterial flora. Bile has a distinct antibacterial power, which indeed is one of its functions in the intestines.

The placenta is an avenue of infection in several diseases: notably small-pox, anthrax, glanders, typhoid fever, and sometimes tuberculosis pass through the placenta from mother to foetus. Streptococci may pass through the placenta of a woman with

ante-delivery sepsis and cause peritonitis in the child. Recurrent fever has been transmitted from mother to foetus, and the specific spirillum has been detected in the latter's blood.

A case has been recorded in which a woman suffering from pneumonia gave birth to a child, which died thirty-six hours afterward, and autopsy revealed a consolidation of the lower left lung, and microscopic examination discovered pneumococci. A hydrophobic cow was delivered of a calf that developed rabies three days after birth.

McFarland divides microbic infection in three heads:

Phlogistic.—Characterized by restricted growth and local irritation.

Toxic.—Characterized by restricted growth and toxin dissemination.

Septic.—Characterized by unrestricted growth in the blood and lymph. In the three groups, the damage is done ultimately, by metabolic products acting on the tissues. If the product be not soluble the harm done is purely local, as in the formation of tubercles by the toxin of the tubercle bacilli.

If the growth be restricted, as in tetanus and diphtheria, the toxin being soluble and diffusible, harm is done to tissues remote from the infected area.

Anthrax and streptococci and other pus organisms by rapid increase in the blood eventually infect all the tissues.

Combinations of these forms of infection may be at first confined to some particular area; the pneumococcus, which is generally restricted to the lungs at the outset, may ultimately infect the blood, causing septicæmia and localized lesions in more or less remote parts, such as the veins of the leg, or inflammation of the meninges. In a topographical sense infection may be local, focal and general. Local disease is limited in extent and at most gives only trifling general manifestations by absorption of products of inflammation, a boil. When an infection becomes well established in a small locality but without active general evidences, it may

still send out a few organisms or small quantities of poison which can attack other parts. Thus from a root abscess, germs may sneak into the blood stream and settle in the kidneys or joint membranes; this is focal infection and is usually subacute or chronic in character. General infection is self explanatory.

Bacteria may become accustomed to the fluids of the body by a similar process and may elaborate free receptors or their own protection, *i.e.*, anti-bacteriolysins (Welch's theory).

In the aged, and in chronic disease of the liver and kidneys, the complement existing in the blood may become reduced in quantity, and the individual may succumb to an infection, which ordinarily would be mild.

Soluble products of bacterial activity which are alkaloidal (basic), crystalline in character, and mostly poisonous, are known as *ptomaines*, or putrefaction alkaloids. They are highly complex in chemical structure, and are difficult to isolate.

The foregoing processes are due to the toxic products of bacteria during their growth in the tissues, substances mentioned before but now deserving a more detailed study from the standpoint of the diseased tissue.

Bacterial endotoxins are poisonous substances liberated only upon the death and disintegration of the germ cells. They are moderately active in attacking wandering and special tissue cells; they are more resistant to heat and ferments than toxins. The identity of these toxins has given rise to considerable dispute and there will be given the two principle ideas concerning them. The older theory considered them integral parts of the cell, peculiar to each virus and calling forth specific responses because of the individuality of each toxin. Recent work has shown that the chemical and immunological response to bacterial injection is similar to that obtained by the use of serum, or egg white or animal cells. For this and more minute reasons it is believed by some that there is in every protein (bacterium, cell, serum) a toxic part with a common construction. Another and peculiar

moiety for each is non-toxic but represents the part which calls forth specific antibodies. The characteristic infectious phenomena of each disease are therefore due to the non-toxic part of its molecular construction. It has been shown that protein cleavage products can cause fever if injected into animals.

Cholera and typhoid organisms do not produce soluble toxins in the body, but when they are disintegrated therein, soluble poisons (intracellular) are liberated.

Bacterioprotoxin or **plasmins** are albuminous bodies produced by bacteria, are not altered by heat, and produce fever and inflammation. The best examples of these are mallein a product obtained from old cultures of glanders bacilli, and the original, or old tuberculin of Koch.

Toxins or **toxalbumins** are soluble, non-crystallizable, non-dialyzable bacterial products which are removable by filtration from the bacteria, and which are thermolabile.

These various poisons produce many of the clinical pathological entities and symptoms, known to physicians. Their highly complex molecular structure enables a group of atoms in the toxic molecule to unite with a certain other group of atoms in the protoplasmic molecule of a body cell. The latter is either killed outright, or else is stimulated to produce other free groups of combining atoms (lateral chains) which may unite with other toxic groups.

Various kinds of cells are attacked in infective processes. Leucocytes may be degenerated, forming pus; red blood cells may be dissolved, causing anæmia; important nerves may be degenerated; or muscle fibers of the heart may undergo fatty degeneration and die. Again, mechanically important serous cavities may be filled with serum, interfering with normal functions of the enveloped organs. The heart orifices may be closed partially or emboli may form, or false membranes block the air passages, and a hundred other pathological changes may be wrought by these toxins.

Most toxins are easily decomposed by sunlight, air, and heat. Absolute alcohol separates the active principles from the bouillon in which it grows. Ammonium sulphate also precipitates the toxins from cultures of tetanus and diphtheria bacilli, from which they may be collected, dried and powdered, and in this state may be kept much longer without deteriorating into inert substances. Small quantities of bile and pancreatic juice destroy the toxic properties of diphtheria and tetanus toxin.

Since the toxins cannot be isolated in a chemically pure form, their exact composition cannot be known, except by studying their effects upon animals and animal tissues. Hence, when anti-toxin, added to toxin in a test-tube is injected into an animal, and no harm results, it is rightly assumed that the toxin is neutralized, and both are chemically bound; yet if fresh toxin is added to the mixture, it is no longer neutral.

If the toxin of the pyocyaneus and the anti-toxin be mixed so that they neutralize each other, and if the mixture is heated, the neutralization disappears, and the mixture becomes toxic again. That the union is a chemical one, may be inferred from the fact that it is more rapid in concentrated solution than in weak, and is much quicker when warmed than when cold, and it follows the law of multiples, 1 part toxin neutralizing 1 part of anti-toxin, and 10 parts of toxin neutralizing 10 parts of anti-toxin. All this is in accord with chemical laws. Toxins sometimes degenerate into what Ehrlich has called *toxoids*, substances that bind (unite with) anti-toxin just as effectively as toxins, while they are not poisonous, yet may stimulate healthy cells to secrete anti-toxins if they are injected into the body of experiment animals.

More is known about the toxins of diphtheria and tetanus bacilli than of any other. Diphtheria toxin has numerous component substances, one of which is the toxin that causes the acute phenomena of diphtheria intoxication. Another, *toxon*, causes cachexia and paralysis some time after infection.

Tetanus toxin is composed of two substances: tetanospasmin and tetanolysin. The first unites chemically with the motor elements of the nervous system, producing degeneration and causing tremendous contractions of the muscles governed by the nerves involved. The second has the property of dissolving tissue, such as blood cells.

Tetanus toxin travels from the infected site to the cord by way of the nerves; it is exceedingly poisonous; a single prick of the finger with a needle moistened with toxin, has induced tetanic symptoms.

If tetanus toxin of known strength is mixed in a test-tube with fresh brain substance of a guinea pig, the toxin is no longer toxic for guinea pigs. This shows that there is a chemical union of the toxin and the cells of the brain. Cells of other organs have no such effect. This explains specific action of tetanus upon nervous tissue.

Aggressins.—If tubercle bacilli are injected into the abdominal cavity of a guinea pig, tuberculosis is produced. If the exudate produced in the peritoneum, consisting of fluid and cells, be sterilized and injected into another guinea pig, together with some virulent tubercle bacilli, the animal will succumb in twenty-four hours. If the exudate alone be injected no effect will follow; if bacilli alone are injected, a tuberculous peritonitis will be produced in a few weeks. It is the exudate plus bacilli that does the harm. The exudate is, in this instance, the aggressin. Bail, who originated the doctrine of aggressins, believes that a bacteriolysin is produced, which, acting on the bacilli, liberates an endotoxin, which paralyzes the polynuclear leucocytes, inhibiting their action as phagocytes.

By heating the exudate to 60°C. the aggressins are increased in activity, and it has been found that small amounts are relatively stronger than larger ones.

This phenomenon has been explained by Bail in this way. He assumes that there are two substances in the exudate, one is

thermolabile, which prevents rapid death, the other is thermostabile and this is favorable to rapid death.

Bail assumes that a tuberculous cavity in an animal contains a great amount of the aggressin, which prevents chemotaxis of the polynuclear leucocytes, but not of the mononuclears or lymphocytes.

In the peritoneal cavity into which tubercle bacilli without aggressins, have been injected, an active phagocytosis at once is begun by the polynuclears, and the injected bacilli are in a great measure destroyed, and those left develop more slowly, producing a tuberculosis in normal course of time. It is possible to immunize animals against this aggressin producing an anti-aggressin, which substance will not only neutralize the aggressin but also stimulate the leucocytes to phagocytosis.

This aggressin theory has been applied to other infections with like results, notably in pneumococcus, typhoid, dysentery, and plague infection.

CHAPTER IV

IMMUNITY

By immunity is understood the inherent power of a living body to successfully withstand the invasion of infective agents, *e.g.*, bacteria, or such deleterious and toxic substances as toxins, drugs, complex poisonous albumins, snake venom, foreign blood sera, etc.

The following tables will, perhaps, be helpful in the study of the subject.

1. Immunity	{ Natural	{ Racial immunity
		{ Inherited immunity
	{ Acquired	{ Active immunity
		{ Passive immunity
2. Immunity	{ Anti-toxic	
	{ Anti-bacterial	

It is a well known fact that one attack of an infectious disease generally protects an individual against a subsequent attack. It has also been known for centuries that the human system, by first taking very small doses, and gradually increasing them, can be so accustomed to poison, that large, and otherwise deadly quantities may be taken at one time with impunity. Among the poisonous substances to which men can accustom themselves are: tobacco, morphia, arsenic, and alcohol. Animals treated in a like manner also become immunized to powerful toxins, snake venom, etc.

Natural Immunity.—The hog is immune to snake venom; the chicken to tetanus. Man is immune to hog, or chicken cholera. The negro is not so susceptible to yellow fever as is the white. Animals cannot be infected with scarlet fever, malaria, and measles. Young adults are more susceptible to typhoid fever than are elderly ones. Infants are exceedingly prone, to suffer from milk infection while older children are not. Again, one

individual may contract a disease, while another exposed at the same time will not. Inherited immunity is exemplified by the history of races into which a new disease was introduced at first with high mortality but later with great reduction in the severity of the infection.

Acquired Immunity.—*Actively acquired* by infection. One attack of yellow fever immunizes the individual against subsequent attacks. Vaccination actively immunizes against small-pox.

Passively Acquired.—Actually injecting protective substances (anti-toxic sera) into the blood. The immunity against a given disease (diphtheria) resides in the anti-toxic sera.

Immunity is nearly always relative. A small quantity of toxin may be innocuous, while a large quantity may cause a fatal toxæmia.

There have been several theories advanced to account for the various phenomena of immunity, the oldest ones, beginning with Pasteur, being that some substances vitally necessary to the virus was used up or that something was retained to affect new microbial attacks.

The modern conception of immunity deals with two theories, the theory of phagocytosis of Metchnikoff, which may be termed the cellular or biologic one, and the lateral-chain, or the humoral or chemical theory of Ehrlich. Both of these are extremely ingenious and explain satisfactorily why certain bacteria are unable to infect the body, and why, the body once infected, cannot, in many diseases, be again infected. Furthermore these theories make it clear to us why the body tissues during life do not fall an easy prey to many putrefactive bacteria, as after death.

Phagocytosis is essentially a theory of cell-devouring. Leucocytes which are white mobile cells of the blood, and other fixed cells, defend the body against infection by devouring the invading agents of disease (Fig. 14).

Metchnikoff considers the subject of phagocytosis under three aspects; (1) nutritional; (2) resorptive; (3) protective.

Amoeba and certain other unicellular vegetable organisms belonging to the *myxomycetes* possessing amœboid properties and having the faculty of throwing out pseudopodia or protoplasmic arms, acquire their food by enveloping smaller organisms and other nutritious matter which they absorb. Certain intracellular ferments, which they possess, digest fibrin and gelatine, and convert starch into sugar. These cells protect themselves against inimical microörganisms by enveloping and digesting them.

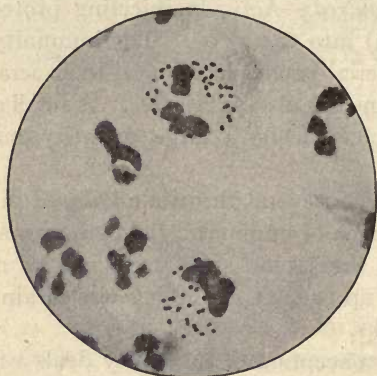


FIG. 14.—Phagocytosis. Gonococci in leucocytes in pus from gonorrhoea. (Kolle and Wassermann.)

They are attracted by food and moisture (called *positive chemotaxis*) and repelled by strong solution of salt, poisons, etc. (*negative chemotaxis*).

Higher in the animal scale among the multicellular organisms, the cells of the intestines have the property of absorbing and digesting food. These fixed cells are called *sessile phagocytes*. Still higher in the scale (man) certain digesting cells are present in the digestive tract, which are incapable of absorbing food. They, however, secrete ferments which digest gelatine and fibrin, and convert starch into sugar. But other cells of the animal body, the leucocytes, large mononuclears and certain fixed tissues-cells, have the property of engulfing foreign bodies like bacteria

and of digesting them; it is to these that Metchnikoff ascribed the protective power of phagocytosis. Cells of a protecting character in man are either microphages or macrophages. The microphages are the polynuclear leucocytes, which are concerned in the protection of the organism against acute infections, the bacteria of which they take up and devour. The macrophages consist of the large lymphocytes, the endothelial cells, and some connective tissue cells, which take up foreign bodies. Both of these classes contain ferments; microcytase being found in the microphages; and macrocytase in the macrophages. The latter absorb connective tissue cells through their particular ferments, and are active in immunizing against tuberculosis. These cells perform various functions in the body. When the tissues are invaded with bacteria, the blood shows an increase in the number of these microphages, which have been called the "hygienic police." Summoned to repel invasion, they leave the lymph stream for that of the blood. All the phenomena of leucocytic emigration in inflammation is a manifestation of positive chemotaxis. During practically all the infections, the peripheral blood contains an excess of leucocytes over the normal amount per cubic millimeter (7,600). In exceptional infections, typhoid fever, influenza, measles, and tuberculosis, there is no such increase, or leucocytosis. In malaria (not a bacterial infection) there is also no leucocytosis.

Metchnikoff has described a process in which the phagocytes undergo what he calls phagolysis. The ferment, *cytase*, is discharged and acts extracellularly, digesting the cell body and freeing ferment which will act against the invaders. Metchnikoff further claims that both phagocytosis and phagolysis, either severally, or in combination, are responsible for natural or acquired immunity.

In the case of acquired immunity, it is supposed that the leucocytes become educated. Regarding the toxins against which animals can be immunized by gradually increased doses, it is held

by him that the educated leucocytes neutralize the poison by their secretions.

In the case of anthrax infection, animals infected with virulent cultures of this organism quickly succumb, without exhibiting any leucocytosis (negative chemotaxis).

If the animal has been previously immunized with attenuated culture the injection of a virulent culture is followed by an enormous outpouring of leucocytes at the site (positive chemotaxis), while if the site of the inoculation in the non-immune animal is examined, only a few leucocytes, and some clear serum will be found.

Toxins, if injected, cause a negative chemotaxis. If tetanus spores are injected into an animal, together with some toxin, the animal rapidly succumbs to tetanus, without evincing any leucocytosis. If the spores are washed free from toxin, and injected, active leucocytosis occurs and the animal survives.

A mixed infection of a highly virulent culture, and a non-virulent one, often hastens the action of the virulent one. It is supposed that the non-virulent bacteria engage the leucocytes, so that these cells cannot cope with the virulent ones.

Phagocytosis thus plays an important part in a protective rôle in natural immunity, but no satisfactory theory has yet been offered in explanation of the protective process in acquired immunity, at least against toxins and other soluble and unorganized poisons.

In order to meet the criticisms arising after Ehrlich's theories. Metchnikoff added to his theory by stating that complement and anti-body are enzymic substances derived from phagocytes.

The **cellulo-humoral** theory claims the attention of most bacteriologists, as the probable explanation of the phenomena of immunity. It is certain that cells, either sessile or mobile, and fluids, are important means of internal defense. In order that this theory may be comprehended, certain well-known properties on formal and artificially immunized serum must be understood.

Alexins.—It has been found by numerous observers, that normal blood serum is germicidal for many bacteria, and peculiarly active substance that is contained in the serum, was called by Buchner *Alexin*. This dissolves bacteria and destroys them. It also destroys the red blood cells of other animals. The alexin of a dog's serum dissolves the red cells of a rabbit; it is therefore *hæmolytic*. It also is *thermolabile*, that is, its properties are destroyed by heat ($55^{\circ}\text{C}.$). It is identical with the *complement* of Ehrlich, and the *cytase* of Metchnikoff.

The *complement*, as it will hereafter be called, takes, as already stated, an active part in bacteriolysis, or bacteria-dissolving, and in hæmolysis, or blood-dissolving, it is present in normal non-immune sera. R. Pfeiffer found that if some serum from a guinea pig immunized against cholera spirilla is injected into the peritoneal cavity of a healthy non-immune guinea pig, with some cholera spirilla, that the latter are agglutinated, and ultimately dissolved, having undergone bacteriolysis (Pfeiffer's reaction). The immune serum alone in a test-tube, with the cholera spirilla does not have this action, but if some normal guinea-pig serum is added to the mixture, an immediate solution takes place, showing that the presence of both the normal serum containing the *complement*, and the immune serum, containing the immune body, or *amboceptor*, are necessary to complete the solution of the bacteria. If the *complement* is heated above $55^{\circ}\text{C}.$ for an hour, solution does not take place, even if the immune serum is present, but, after heating the mixture, it may be *reactivated* by adding some fresh unheated *complement*. The complement is thermolabile, *i.e.*, destroyed by heat.

The immune serum is not affected by heat, and is therefore called thermostabile.

These various reactions may be expressed concretely thus:

Bacteria + immune body = no solution.

Bacteria + complement = no solution.

Bacteria + immune body + complement = solution (Pfeiffer's reaction).

Bacteria + immune body + complement (heated) = no solution.

Bacteria + immune body (heated) + complement = solution.

The same phenomena have been observed in the blood of animals immunized against the red blood corpuscles of another animal of foreign species.

If a rabbit is immunized with the blood of a dog by repeated and increasing doses, the serum of that rabbit will become *hæmolytic* to the corpuscles of the dog's blood if they are mixed, provided some normal rabbit's blood complement is added to the mixture.

Dog's erythrocytes + immune rabbit serum = no solution.

Dog's erythrocytes + immune rabbit serum + complement = solution.

Dog's erythrocytes + immune rabbit serum + complement, heated = no solution.

The immune body acts as a preparer of the corpuscles, or bacteria, so that the complement can act upon the cells. The reaction is very like the action of pepsin on fibrin. Hydrochloric acid must be present.

(1) Pepsin + fibrin = no solution or lysis.

(2) HCl + fibrin = no solution or lysis.

(3) Pepsin + HCl + fibrin = solution or lysis.

The HCl corresponds to the immune body.

In the case of hæmolysis, or bacteriolysis the action of the immune body is specific. The immune body of cholera spirilla will not prepare, or fix typhoid bacilli, so that they can be acted upon by the complement. Nor will the immune body of dog's erythrocytes prepare those of a pig, so that the complement may act on them.

A loose chemical union takes place between the bacteria and the immune body, but no such union occurs between the comple-

ment and the bacteria. The same chemical union occurs between the red cells and the immune body in hæmolysis, but not between the cells and the complement.

Ehrlich holds that there are many complements, each one different from the other, and that their action is specific for the different kinds of bacteria or cells with which an animal may be immunized. Bordet and Buchner, on the other hand, maintain that there is but one complement.

The solution of any cells by immune bodies, or anti-bodies, as they have been called, is known as cytolysis. And cytolysins may be produced by making anti-bodies of nerve cells, leucocytes, epithelial cells, liver cells, as well as blood cells, by immunizing an animal against these different cells with repeated injections of the cells or emulsions of them.

Agglutinins are peculiar bodies which have the property of causing certain cells to agglutinate. One of the earliest manifestations of immunity of a certain serum to bacteria, or to blood cells, is this peculiar action of the serum causing either the bacteria or blood cells to clump together in masses. Part of Pfeiffer's reaction is the agglutination of the cholera spirilla in clumps before they are dissolved by the complement and immune body.

Recent studies accord to agglutinins one of the most important places in the defense against disease. Their action is believed to facilitate that of lysins and opsonins, helping the latter by fixing a group of bacteria so that they may be the better prepared for phagocytosis.

If the serum of a typhoid fever patient is mixed, even in high dilutions with some typhoid bacilli, the latter are clumped in isolated groups. Clinically this is known as the *Widal* reaction, and is the most reliable single sign of typhoid fever.

These agglutinins may be produced artificially by injecting large and increasing doses of bacteria into animals. After a time, in the serum of the rabbit, there develops a peculiar body which agglutinates typhoid bacilli, if they are brought in contact with

it. Sera can be rendered so highly agglutinative as to produce this reaction even if diluted 100,000 times or more.

If an animal is immunized against spermatozoa, or the red blood cells of a foreign species, its serum becomes agglutinative to these cells.

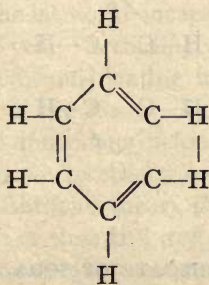
Precipitins.—If a rabbit, or any other animal in fact, is immunized by repeated injections of foreign protein (blood, bacterial culture, etc.) peculiar bodies develop in its blood serum called precipitins, and these can be demonstrated by adding to the serum of the immunized animal in a test-tube a minute portion of the material against which the animal was immunized. As soon as the immunized serum and the specific substance are mixed, a precipitate forms. This is another phenomenon of immunity, and is of more than theoretical importance in medicine. The reaction is strictly specific; thus, if the serum of a goat is injected into a rabbit repeatedly the rabbit's blood will form a precipitate with normal goat's serum if the two are mixed in a test-tube. Old dried blood, semi-putrid blood, blood on white-wash, or rusty steel, even in minute quantities, if dissolved in salt solution, may be used to produce this reaction. In medico-legal matters, this test is of use for the identification of human blood. By some, the phenomenon of agglutination is supposed to be due to the formation of a precipitin, in the meshes of which bacteria or blood cells are caught and agglutinated, and that agglutination is but a modification of the formation of precipitins.

Anti-toxin formation is also another phenomenon of immunity. If an animal, such as a horse, receives numerous increasing doses of a given toxin, say that of tetanus, it, in a short time, becomes so accustomed to the poison, that it can withstand the administration of immense doses. (If these large doses had been given at first, they would have proved fatal.) If the horse is then bled, and its serum injected into rabbits or guinea pigs, they may receive shortly after, at one dose, enough toxin to kill ten such animals. The horse serum thus protected these animals against the toxin, as it was anti-dotal, or in other words anti-toxic. A chemical

union occurs between the toxin and the anti-toxin, since, according to the law of multiples, a definite amount of anti-toxin unites with a definite amount of toxin. If ten times the amount of anti-toxin is used it will exactly neutralize ten times the amount of toxin, and the mixture becomes inert. Again, the union of the two substances follows well-known chemical laws, whereby chemical union takes place more rapidly in concentrated than in dilute solutions, and when the solutions are warm. If the mixture of toxin and anti-toxin is heated, it, instead of being neutral, becomes toxic again. This toxicity can be neutralized again by the addition of fresh unheated anti-toxic serum (*reactivation*).

The production of bacteriolysins, cytolsins, agglutinins, precipitins, and anti-toxins are manifestations of the activity of the immunized organisms. To further understand this activity, Ehrlich's *side-chain* theory of immunity must be comprehended. This is known as the chemical theory. To understand it fully some consideration must be given to the study of the toxin molecule. Ehrlich believes that each molecule of toxin is made up of two groups of atoms, constituting what is known in chemical nomenclature as lateral chains. Many molecules are made up of a central body and lateral chain of atoms which are free to combine with other groups of atoms without disturbing the central body.

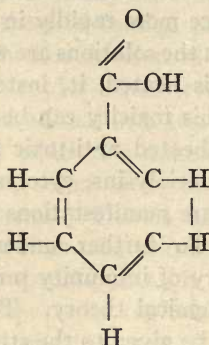
The benzol ring is very suitable for the demonstration of the relationship of the side chain to the central body.



BENZOL.

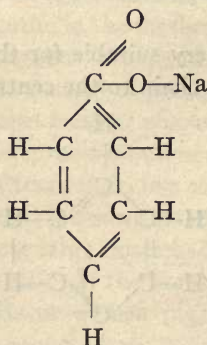
The benzol molecule C_6H_6 is here represented graphically as a ring with a central nucleus of C_6 with lateral chains of H. connecting each atom of C.

If one of these lateral chains H. is supplanted by the acid radical $COOH$. the benzol is converted into *benzoic acid* and its formula is represented thus:



BENZOIC ACID.

If to this acid radical of the benzoic ring, sodium hydroxid unites, supplanting an H in the OH of this radical, we have, instead of benzoic acid, *benzoate of soda*.



BENZOATE OF SODA.

It is thought that as the soda is brought in contact with the central nucleus of the benzol ring, so foodstuffs unite with the central body of the cell molecule in the organism and nourish it.

In the case of toxin, the two lateral chains of its molecule are called *haptophores* and *toxophores*. The haptophores seize the lateral chains of the cell and the toxophores poison it.

Ehrlich conceived that cells were nourished by their lateral chains, each having a central nucleus with many lateral chains called *receptors* bristling all over it. Complex albumins, foodstuffs or poisons (as the case may be) unite with it. This means a chemical union of a part of a cell with all or part of a group of atoms. But certain body cells are only capable of uniting with certain toxins. It is known that the toxin of tetanus has a chemical affinity for the nervous system and for its neural elements and not for liver or spleen cells. The poisons of snake venom seem incapable of uniting with any cells of the pig; therefore, this animal is immune to snake venom.

Now, as these toxins unite with the cells by means of the receptors, the cell is stimulated to produce an excessive number of these receptors, which are cast off and become free. Nature is very prodigal and whenever any of the tissues of the body have been injured, or there is a deficiency, an enormous excess of reparative cells is produced. Weigert first called attention to this phenomenon, which has been called Weigert's *overproduction theory*. So when the haptophores of the toxin molecule combine with the receptors of the cell, the latter are incapable of any further union and are useless to the cell. Accordingly a great number of free receptors are generated, and floating in the blood, engage the haptophorous portion of the toxin. Thus the toxophore is neutralized and rendered innocuous before it can reach the cell. These free overproduced receptors constitute the anti-toxin. This is the essence of Ehrlich's theory (Fig. 15).

Through the process of time and oxygenation the toxophorous group in the toxin becomes innocuous, and only the haptophorous

group remains active; nevertheless the haptophorous group is able to combine with the receptors and to stimulate the cell into generating free receptors. This attenuated toxin is called by Ehrlich *toxoid*. The receptors have been compared to a lightning rod, which if placed within a building would, if struck, cause disaster, while the same rod placed outside of the building, is a means of protection to the structure against lightning. This

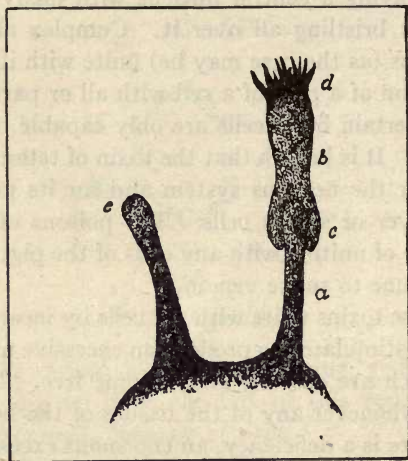


FIG. 15.—*a*, receptor on cell; *b*, toxin molecule; *c*, haptophorous portion of the molecule; *d*, toxophorous portion; *e*, receptor. (Williams.)

theory can be applied to the production of other anti-bodies. If blood cell, bacterial cell, or any animal fluid possessing a haptophore is capable of combining with side chains (receptors) of the cells of the immunized, just as a key fits a lock, then the cells are stimulated to produce excessive numbers of receptors, and these constitute the anti, or immune body. It is possible to produce from rennet, egg-albumin, cow's milk, and from many other albuminous substances, immune bodies by injecting these substances into animals (Figs. 16, 17).

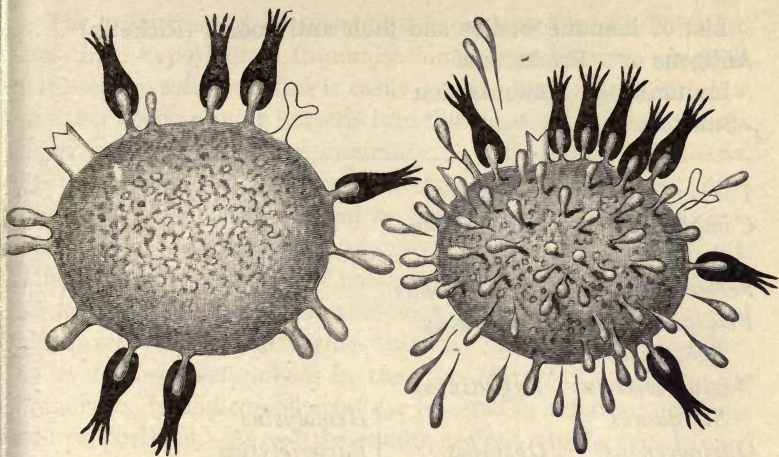


FIG. 16.—EHRlich's LATERAL-CHAIN THEORY. Cell with numerous receptors of various kinds and shapes to which are united the toxin molecule. Note the free receptors.

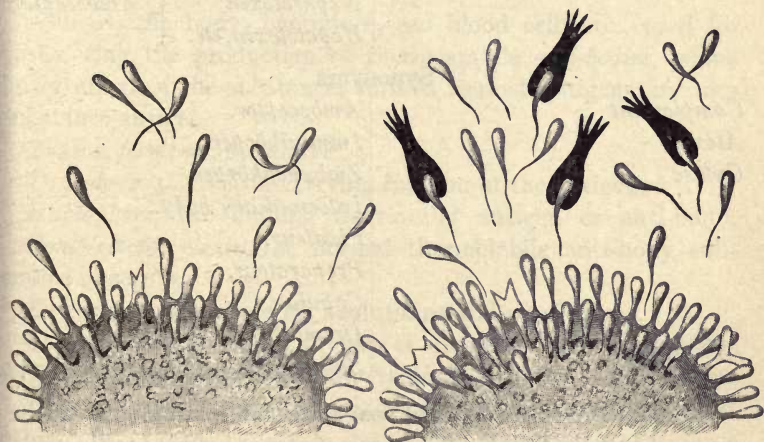


FIG. 17.—EHRlich's LATERAL-CHAIN THEORY. In one figure the free receptors (anti-bodies) are united with the toxin molecule, the attached receptors have no haptophores united to cell.

List of immune bodies and their anti-bodies (Ricketts)

Antigens or Immunizing Substances	Products of Immunization
---	-----------------------------

<i>Toxins</i>	<i>Anti-toxins</i>	
<i>Complements</i>	<i>Anti-comple- ments</i>	
<i>Ferments</i>	<i>Anti-ferments</i>	
<i>Precipitogenous Substances</i>	<i>Precipitins</i>	
<i>Agglutinogenous Substances</i>	<i>Agglutinins</i>	
<i>Opsinogenous Substances</i>	<i>Opsinins</i>	<i>Hemolysins</i> <i>Bacteriolysins</i> <i>Special cytotox- ins Such as</i> <i>Spermotoxin</i> <i>Nephrotoxin</i> <i>Hepatotoxin, etc.</i>
<i>Cytotoxin Produc ing Substances</i>	<i>Cytotoxins . . .</i>	
		<i>Consisting of two bodies Complement Amboceptor</i>

Synonyms

<i>Complement</i>	<i>Amboceptor.</i>
<i>Alexin.</i>	<i>Immunkörper.</i>
<i>Cytase</i>	<i>Zwischenkörper.</i>
	<i>Intermediary body.</i>
	<i>Fixateur.</i>
	<i>Preparateur.</i>
	<i>Copula.</i>
	<i>Desmon.</i>
	<i>Substance sensibilisatrice.</i>

It is well known that rennet coagulates milk, but if some of the serum of an animal immunized against rennet is added to the milk, the latter cannot be coagulated because the anti-rennin combines with the rennet and renders it inert.

The production of bacteriolysins is explained by Ehrlich's lateral-chain hypothesis. Immunization against bacteria which do not produce soluble toxins is easily secured by repeated injection of either dead or living bacteria into the organism. It is not easy, however, to confer passive immunity, as in the case of diphtheria, by the injection of the serum of the immunized animal. The immune body is alone present in the serum generally and some complement must be added to effect bacteriolysis. The serums which aid in the solution of bacteria are known as anti-bacterial serums, which, though not anti-toxic, may check invasions and aid in recovery by destroying bacteria. It is possible to effect an *in corpore* bacteriolysis in the case of typhoid fever if the immune body and complement are injected in sufficient amounts and proportions. As yet the results are not satisfactory from a clinical standpoint.

A study of Fig. 18 will show clearly the exact combinations of various substances engaged in the immunity process. Some of the terms must be defined.

Antigen, the body, bacterium, red blood cell, etc., used for stimulating the production of thermostabile *anti-bodies*, which latter are then the substances formed against antigens; inciting substance-antigen.

Toxins, ferments, see above.

Toxophore, the poison-carrying fraction of the antigen.

Haptophore, the binding fraction of antigen or anti-body.

Complement, alexin the normal thermolabile anti-body substance in serum.

Zymophore, toxophore for agglutinins and precipitins.

Cytophile fraction is that part of anti-body which combines with cell, while *complementophile* fraction joins with complement.

Immune body, the thermostabile anti-body against bacterial or other cells.

By immunizing with complement or anti-body we obtain respectively anti-complement and anti-immune body which

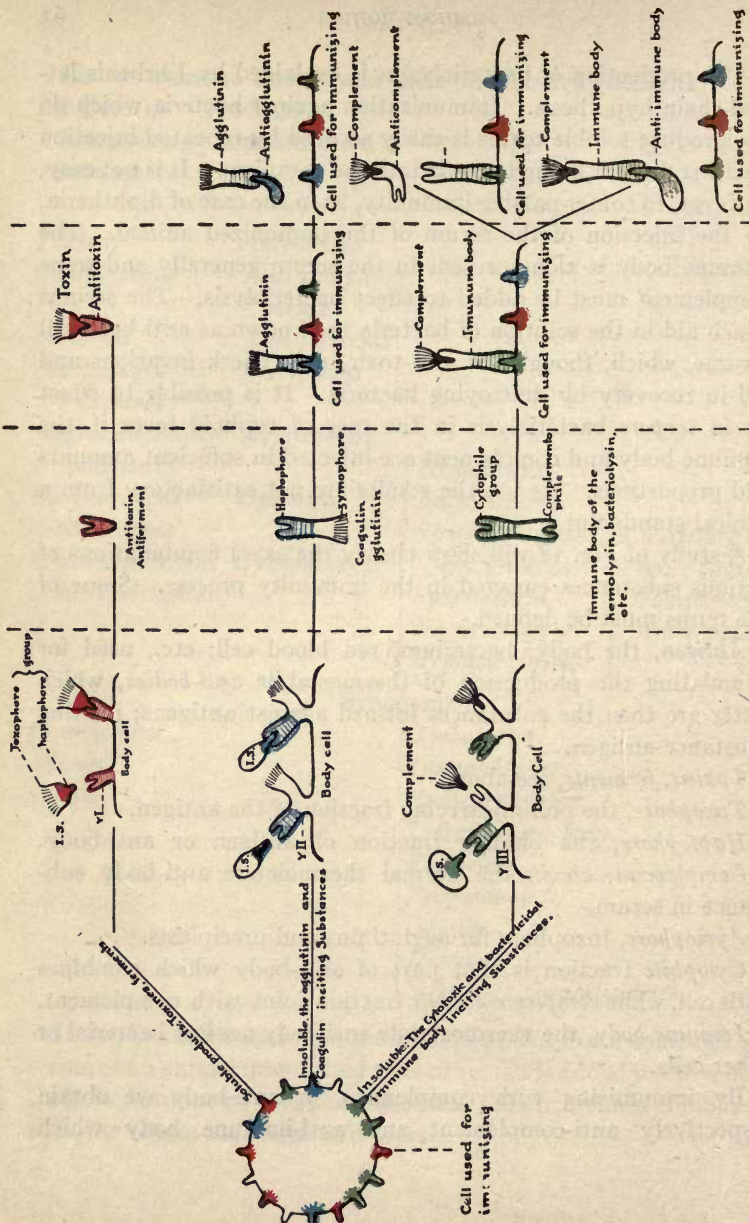


FIG. 18.—The structure of cell receptors and immune bodies, according to Ehrlich's conception. (From Hiss and Zinsser's bacteriology. Copyright by D. Appleton & Co.)

experimentally will neutralize the action of these two substances. The complement being the really responsible potent factor in all these reactions it may be assumed to have two binding affinities, one to the cells which it designs to help and another effect upon antigen. If the former be absorbed in any abnormal manner the latter is valueless.

Cell Receptor and Immune Bodies (follow Fig. 18).—First Order: Simple union of toxins (soluble) and fixed or free receptors or antitoxins; no complement needed.

Second Order: Concerns agglutination and precipitation. Antigen has two affinities, one for the haptophore of anti-body, another for the agglutinin of the anti-body. The anti-body must therefore have reversed corresponding fractions. The zymophore of anti-body acts when the two haptophores have united and produces the agglutination or precipitation. No complement is needed.

Third Order: Concerns bacteriolysins, hemolysins or bacterioly-sins, etc.; have haptophore for anti-body, and a toxophore. Anti-body has haptophore for antigen and for the haptophore of the complement. The union of the three must occur. Complement is necessary for the destruction of the bacteria which it accomplishes through its zymophore.

Anaphylaxis.—Against protection, the opposite of prophylaxis; also called Hypersusceptibility. This phenomenon, first described by Theobald Smith, Portier and Richet, consists in a condition of extreme sensitiveness of animals against foreign proteins. If a guinea pig be injected into the peritoneum with a minute quantity, say $\frac{1}{1,000}$ gram, of horses' serum and eight to ten days later receive a quantity of $\frac{1}{10}$ gram, the animal will become uneasy, then depressed, have dyspnea, scratch itself violently about the face and finally die after an intensification of these symptoms. Similar symptoms have been observed in persons receiving diphtheria anti-toxin therapeutically. The condition of high sensitivity to this anti-toxin is called *allergie* and upon its degree depends the reaction following anti-toxin admin-

istration. The skin eruptions, joint pains and edema of serum sickness are also evidences of this condition. It is said that those persons who suffer after anti-toxin are susceptible to the emanations from horses and the physician should make inquiries in this direction when contemplating the injection of all sera.

In experimentally induced hypersusceptibility the reaction is specific. The condition is transmissible from mother to foetus and it can be transferred from adult to adult passively by injecting the blood of a sensitive animal into a normal one. The first dose is called the sensitizing one, the second the intoxicating. The incubation period of the sensitization varies with the nature of the protein; for horse serum it is from eight to twelve days, for bacterial proteins from five to eight days. The sensitive period may last for several years. In searching for the cause of this reaction it was found that there are (1) a spastic distention of the pulmonary alveoli probably both of central and local nature, (2) scattered hemorrhages in the organs and (3) hemorrhages with ulcerations in the gastric mucosa. There have been many theories for this phenomenon, but those of Vaughan, Friedberger and Wolff Eisner may be condensed and compounded about as follows. The body is unprepared to care for parenterally (otherwise than gastrointestinal tract) introduced protein and must develop anti-body or enzyme to care for it. This enzyme or anti-body works slowly and carefully disposes of the foreign protein, the products of which are slowly absorbed and removed. In accord with the overproduction theory this anti-substance is in large quantity when another introduction of protein occurs, and goes to its work with avidity so that it rapidly breaks the protein up into toxic elements which cannot suddenly be cared for by the body. It is also thought by some workers that the body protein of the animal in question is attacked and split, liberating toxic fractions, since the injected protein would not be adequate in amount to accomplish poisoning. These protein toxins attack nervous and parenchymatous tissues. Another very plausible theory would have it

that the first injection directly sensitizes important cells which are destroyed when an intoxicating dose arrives.

The similarity of characters between serum shock and immediate traumatic shock was thought to shed some light on the subject. The latter is believed to be due to protein degradation products, due to trauma or low blood pressure, which have a paralyzing effect upon unstriated muscle or upon the vasomotor centers.

It has been shown that an anti-anaphylactic state can be produced by repeated small injections of protein at intervals too short to allow incubation of an intoxicating dose. Use is made of this knowledge in the case of persons who need anti-serum but who show sensitivity to it. Repeated small but increasing quantities are put into or under the skin or into the vein until the patient can receive without reaction the full dose. This is called desensitization.

Friedberger has used these facts to elaborate a theory of infection. He believes that bacteria circulating in the body stimulate anti-bodies, combine with them and that when complement acts upon this union toxic substances are set free.

In explaining all infectious diseases on this basis one assumes that sometime in life a person has been sensitized by bacteria or their proteins so that he is receptive for a virulent germ when this has overcome the primary external bodily defenses. It is also to be considered the modern explanation of diathesis.

McKail divides anaphylaxis as follows:

Natural Anaphylaxis, depending upon

- (a) Species of animal, for example cholera in man, anthrax in cattle, glanders in horses.
- (b) Age—diphtheria in children, erysipelas in the elderly.
- (c) Individual—to white of egg, or blood serum, even by ingestion ("one man's meat is another man's poison").

Acquired Anaphylaxis, depending upon

- (a) An attack of disease, erysipelas, diphtheria.
- (b) The injection of dead cells, tuberculin.
- (c) Injection of nitrogenous matter, blood serum and egg-white.

Direct practical application of theoretical speculation about hypersensitivity may be made in explaining, treating and preventing certain states in man. Mention has already been made of the possibility of intoxicating persons susceptible to the presence of horses by the injecting of horse serum. The only treatment for such a "Schock" is an injection of epinephrin or atropin.

Serum sickness is explained as a digestion of foreign serum still in the body as such when sufficient ferment has accumulated to digest it, a period of three to twelve days. Whether this be correct or not, symptoms can be made milder or prevented by dividing the doses by some hours. A state of hypersensitivity or allergie may also exist to pollen of plants, certain foods and drugs, dusts of feathers and hair or dandruff from cats and dogs. The evidences of this state take the form of asthma, gastrointestinal disturbances, and skin eruptions of which urticaria and eczema are the commonest. Detection of this hypersensitivity may be subjective at times but usually it has to be established by technical means. It so happens that allergic persons have either a definite anti-body reaction or the local sessile receptors in the cutaneous cells are stimulated, for the application of the responsible protein to an abraded area on the skin will produce at the point a swollen red areola. In the cases of infection of a bacterial or toxic mixture this reaction usually requires twenty-four hours to develop, a delay suggesting that anti-bodies are involved. In the case of serum or pollen allergie, the reaction is almost immediate, suggesting local cellular preparation. This is the basis of skin tests, except with tuberculin and the Schick test. Prevention of such allergie poisonings is best accomplished by the avoidance of

the offending material. Treatment and prophylaxis take the form of injecting solutions of the responsible protein under the skin. For example ragweed is the most common cause of hay fever. Solutions of its pollen, made in standard dilutions of 1-10,000 to 1-500 are injected in rising quantities, beginning with the weakest. Treatment should be given during the winter and spring so that as high a degree of desensitization as possible will be accomplished. Little can be expected in the hay fever season.

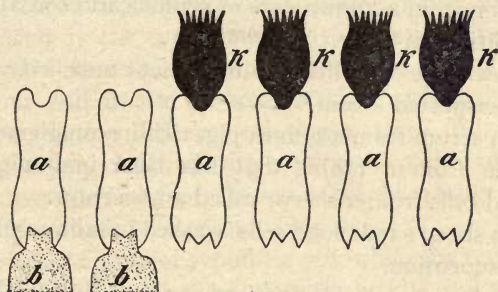


FIG. 19.—Illustrating the conception of deviation of complement. *a*, Amboceptor; *b*, antigen; *k*, complement. (MacNeal.)

Complement Fixation.—Hemolysis occurs when the serum of a rabbit immunized against washed sheep's red blood cells is mixed with fresh washed sheep's corpuscles in the presence of complement. If, however, complement be absorbed in any way, a solution of the coloring matter of the red cells will not occur in this mixture. Complement will combine with anti-body in the presence of antigen. This fact has been taken advantage of in determining both the nature of antigen and the presence of anti-body. Its most important practical use is in syphilis, to the diagnosis of which Wassermann applied it, and the Wassermann test is for the presence of syphilitic anti-body in the blood serum of syphilitics.

This test is positive from the initial lesions all during life unless the patient has been successfully treated. Indeed the parasyphilitic states also give it. The principles of the test are also used for determining the presence of tuberculous, leprosy, typhoid and other anti-bodies.

The materials necessary in the Wassermann test are as follows:

1. Syphilitic antigen, extract from the syphilitic liver of a foetus, in alcohol, ether or water; lipoids like lecithin or extracts from guinea pig's heart will act as antigen.

- 2a. Serum from a known case of syphilis and containing therefore syphilitic anti-body.

- 2b. Known non-syphilitic serum without anti-body.

3. The suspected serum.

4. Fresh serum from a guinea pig, rich in complement.

5. Serum from a rabbit that has been immunized against washed red cells from a sheep; called amboceptor.

6. Fresh sheep's red blood cells, washed in saline and made into 5 percent suspension.

The solutions are all standardized so that only sufficient of each is added to complete the absorption or produce the hemolysis. The serum known to be syphilitic and the suspected serum are heated to 56°C . for thirty minutes to destroy the native and inherent complement. The rabbit anti-sheep cell serum is also heated to this degree.

The hemolytic series, *i.e.*, sheep's cells, rabbit's anti-sheep's cells serum and complement are standardized to find out what quantities will exactly complete hemolysis. These quantities are the *units*. It is necessary to control tests to find out what quantity of the antigen and known syphilitic anti-body will unite to bind the determined quantity of complement. The tests are performed in small tubes so as to have a long column of fluid easier to observe. Tubes are set as follows:

A. 1 unit #1 + 1 unit #2a + 1 unit #4.

B. 1 unit #1 + 1 unit #3 + 1 unit #4.

C. 1 unit #1 + 1 unit #2b + 1 unit #4.

D. 1 unit #2a + 1 unit #4.

E. 1 unit #2b + 1 unit #4.

F. 1 unit #3 + 1 unit #4.

G. 1 unit #4.

H. 1 unit #1.

J. 1 unit #2a.

K. 1 unit #2b.

L. 1 unit #3.

+ 1 unit #5 + 1 unit #6 = No hemolysis.

+ 1 unit #5 + 1 unit #6 =

if #3 be Syphilitic, no hemolysis.

if #3 be Non-syphilitic hemolysis.

+ 1 unit #5 + 1 unit #6 = Hemolysis.

+ 1 unit #5 + 1 unit #6 = Hemolysis.

+ 1 unit #5 + 1 unit #6 = Hemolysis.

+ 1 unit #5 + 1 unit #6 = Hemolysis.

+ 1 unit #5 + 1 unit #6 = Hemolysis.

+ 1 unit #5 + 1 unit #6 = No hemolysis.

+ 1 unit #5 + 1 unit #6 = No hemolysis.

+ 1 unit #5 + 1 unit #6 = No hemolysis.

+ 1 unit #5 + 1 unit #6 = No hemolysis.

The tubes receive first the solutions on the left and are placed in the 37°C. incubator for two hours to allow union of their various parts, particularly the complement with others. They then receive the solutions on the right, are placed in the incubator for half an hour and in the ice-box overnight, when they are examined for a solution of the red coloring matter. If it occurs, the column is perfectly clear red with some residue of extracted cells. If no hemolysis has occurred, the red cells form a layer at the bottom, and the column is clear and colorless.

A and B are the tests of syphilitic sera while the remaining are to find out if the other solutions affect the results of A and B. Of course tube G represents simply the complete hemolytic system. The extra tests are to exclude the possibility of interference on the part of any single member with the complement No. 4. *The character of the test is found in tube A where syphilitic antigen and serum have bound or fixed the complement so that it cannot unite with the rabbit serum and sheep's corpuscles to hemolyze the latter. This is a positive test. A negative test is when hemolysis occurs, since no anti-body is present to unite with complement in the presence of antigen.*

Complement Deviation.—This is a condition arising when there is too much amboceptor and too little complement. The free amboceptors adsorb complement and there is none left for cell needs or renewed demands. It is to be distinguished from complement fixation. The terms are not interchangeable.

ANTI-TOXINS, VACCINES, AND TOXINS

The following is Wassermann's list of anti-toxins:

Anti-toxins for bacterial toxins:

Diphtheria

Tetanus

Botulism

Pyocyaneus

Symptomatic Anthrax

Anti-leucocidin, an anti-toxin against the leucolytic poison of staphylococcus

Anti-toxins for the blood dissolving toxins of certain bacteria.

Anti-toxin for animal toxins:

Anti-venene for snake venom

Anti-toxin for spider poison

Anti-toxin for scorpion poison

Anti-toxins for certain poisons in fish, eel, salamander, turtle, and wasp sera.

Anti-toxins for plant poisons:

Anti-ricin for castor-oil poison

Anti-abrin for jequerity bean poison

Anti-robin for locust bean poison

Anti-croton for croton-oil bean poison

Anti-pollen for pollen of plants that produce hay-fever.

There may be added to this list a number of anti-sera developed to a practical value in recent years whose activity is dependent not on anti-toxin content, but upon their ability to agglutinate, precipitate, and opsonify their respective microorganisms. These sera are made against infections with:

Pneumococcus

Meningitis coccus

Streptococcus

Plague

Dysentery (bacillary)

Staphylococcus

Typhoid fever

The first two have proven of more definite value than the rest.

Manufacture of Anti-toxins.—If small doses of a given poison, such as diphtheria toxin, be repeatedly injected into a susceptible animal, and if the dose is gradually increased, there appears, after a time, in the blood serum, an anti-body, or anti-toxin. This substance in the serum is secreted by the cells and corresponds to the free receptors in Ehrlich's lateral-chain theory. If an animal be injected with the anti-toxin, and then with a large dose of toxin—say ten times the amount necessary to kill it if it had not received the anti-toxin—it will not be harmed. Here the free receptors artificially supplied to the animal unite with the haptophorous chains in the toxin molecule, and naturalize, or bind, the toxophorous or poisonous chains in the molecule, and prevent toxophore from attacking important vital cells belonging to the animal. And if the anti-toxin and toxin, after being mixed in a test-tube, are injected into a susceptible animal, no harm results, if they are in proper proportions, since the same thing has happened *in vitro* that happened in the animal, the receptors and haptophores have united; the toxophores are bound, and the animal is unharmed.

The manner of making the **diphtheria anti-toxin** can be taken as a type.

Diphtheria bacilli are grown for seven to ten days in .1 percent dextrose bouillon at 37°C.; as the bacilli grow they elaborate a very powerful poison or toxin, which is highly complex in composition. Strains that habitually grow on the surface of the bouillon are used, the access of air enhancing the production of toxin. It is easily decomposed by heat, light and oxygen, and should be used soon after it is prepared. After the cultures have grown for several days, the bouillon is filtered through a porcelain filter, and is then stored in sterile bottles in an ice chest. Horses are generally immunized, since they are susceptible to the action of the toxin, and are easily managed. Before being used they are carefully tested with tuberculin for tuberculosis and with mallein for

glanders. Being very susceptible to infection with tetanus while undergoing treatment, a prophylactic injection of *tetanus anti-toxin* is given each animal. McFarland found that the death rate from tetanus, in a large stable, was greatly reduced after using tetanus anti-toxin as a prophylactic measure.

Immunization is started by injecting into a previously examined healthy horse a mixture of toxin and anti-toxin in which the former is not quite neutralized by the latter. These quantities are determined by guinea-pig tests. Such a mixture is safer for the horse and begins the immunity reaction more promptly. A few doses like this are given after which pure toxin is used. This is followed by a rise of temperature, local reaction and systemic disturbance. After waiting for all reactions to disappear injections are continued by slow increases, until, after a few weeks or months, 1,000 c.c. of toxin are injected at one time (enough to have killed a dozen horses that had not received the smaller doses previously). The injection of the toxin is followed by an immediate fall in the anti-toxic power of the serum, only to be followed by a quick rise. The horse will not produce anti-toxin indefinitely. After the animal has been immunized sufficiently, his blood is drawn from the jugular vein, and after the clot has formed the serum is drawn off and stored.

Anti-toxins are found to lie in the pseudoglobulin fraction of the serum. Even though an anti-serum be strong, a large quantity would have to be injected to obtain high unit value. The globulins of the whole blood are now precipitated by 30 percent ammonium sulphate and the pseudoglobulins are dissolved by normal salt solution. This concentrates high unit values into small bulk. When removed from the horse, serum may contain 300-800 units per cubic centimeter. After concentration a value of 3-10,000 units may be obtained.

McFarland found that a horse was capable of producing enough anti-toxin to protect 806 other horses against doses of toxin, each one of which was equivalent to the total amount of toxin that the

immunized horse received. Thus there is evidently a tremendous overproduction of anti-toxin far above the needs of the animal.

The various component parts of the toxin stimulate the cells of the horse to produce the receptors, or anti-toxin. The toxoids, themselves not poisonous, have the property of stimulating the production of anti-toxin. We measure the anti-toxic powers of the anti-toxin with units arbitrarily devised. *An anti-toxic unit is that amount of horse serum just necessary to protect a 250-gram guinea pig against 100 times the minimum lethal dose of toxin.*

To standardize anti-toxin, we must employ animals, into the bodies of which toxins and anti-toxins are injected. If a certain amount of anti-toxin is necessary to protect a guinea pig against ten times the minimum fatal dose of toxin per 100 grams of guinea-pig weight, then we know that the anti-toxin contains so many units. *The minimum lethal dose of toxin is the smallest quantity that will kill a guinea pig of 250 grams in four days.*

A standard anti-toxin is kept by governments to be used as a control of the products of biological chemists.

Against this standard anti-toxin a toxin of unknown strength is measured by means of guinea pigs. The toxin unit thus found is then used to determine the anti-toxic unit of anti-toxins of unknown power.

Anti-toxic serum is preserved by the addition of .5 percent of tri-cresol or phenol. It remains practically unchanged in strength for a year or more.

It is not only of value as a curative agent, neutralizing the toxins already formed, but is valuable as an immunizing one against infection. Persons exposed to diphtheria and giving a positive Schick test should be given an immunizing dose of 1,000 to 1,500 units. If injected early in a case of diphtheria, it is much more likely to do good, than if used later. Some desperate cases have received 100,000 units and have recovered. The following is the very good guide given by Park:

SINGLE DOSE ONLY

Infant, 10 to 30 pounds (under 2 years)

Mild	Moderate	Severe	Malignant
2,000	3,000	5,000	
3,000	5,000	10,000	10,000

Child, 30 to 90 pounds (under 15 years)

3,000	4,000	10,000	10,000
4,000	10,000	15,000	20,000

Adults, 90 pounds and over

3,000	5,000	10,000	15,000
5,000	10,000	20,000	40,000

Method of Administration

Intramuscular

or

 $\frac{1}{2}$ Intravenous

and

Subcutaneous Intramuscular $\frac{1}{2}$ Intravenous

or

or

and

 $\frac{1}{2}$ Intramuscular

Intramuscular

Subcutaneous

 $\frac{1}{2}$ Intramuscular

or

Subcutaneous

Subcutaneous

Tetanus Anti-toxin.—Tetanus anti-toxin is produced in a manner similar to that of diphtheria anti-toxin. As the horse is exceedingly sensitive to tetanus toxin, before the immunizing process is begun, the toxin is attenuated by heat or iodine.

The anti-toxin is standardized, as in diphtheria, by testing its potency against the toxin. A guinea pig of 500 grams weight is used, and test toxin is employed of such strength that .01 c.c. will kill the guinea pig in about four days. The United States unit of tetanus anti-toxin is now the least quantity of anti-tetanic serum necessary to save the life of a 350-gram guinea pig for ninety-six hours against the official test dose of standard toxin furnished by the Hygienic Laboratory of the Public Health Service.

The toxin of the tetanus bacillus has such an affinity for nervous tissue that, once it becomes attached, separation is difficult, or even impossible if union has existed for some time. More than this the toxin has a greater affinity for nervous tissue than for its

own anti-toxin. If an animal be injected intravenously with anti-toxin and intracerebrally with toxin it will die of tetanus. This explains why the use of anti-toxin in the treatment of the disease must be vigorous, early and direct. Dosages of 10-50,000 units are necessary before symptoms have become well established. Subcutaneous methods are too slow and indirect. Anti-toxin should be given into the spinal canal, into the vein and around the wound. Prophylactically anti-serum should be given in every case of penetrating, lacerated wound especially if soiled with earth, street dirt, rust or gun waddings. Tetanus antitoxin is more efficient as a prophylactic than as a remedy.

Streptococcus Antiserum.—The three principal groups of streptococci, hemolytic, non-hemolytic and viridans, have different immunity factors and each kind produces its own anti-bodies. These however are never in great amount and this seems due to the rather feeble antigenic power of the cocci themselves. The only useful anti-sera are those prepared against a large number of strains of each of the varieties by repeated injections over a long period. Their antigenic fractions seem to be hemolytic, leucocytolytic and neurolytic. The anti-bodies formed in horse's serum seem to decrease in value after removal from the body. They are chiefly agglutinative and anti-toxic. For clinical use the serum should be as fresh as possible and used intravenously in doses of 50 to 200 c.c. It has been employed in endocarditis, osteomyelitic and puerperal septicemia with some promise.

The **Anti-pneumococcus** serum is prepared in the same way. Horses are immunized by the injection of first autolysates then living cultures, and the horse's blood, after a period of treatment by cultures, is drawn off, preserved with tri-cresol. It is used in the crude form of serum as the anti-bacterial powers are not easily concentrated. It is standardized so that .2 c.c. shall protect a mouse against 100,000 times the amount of a culture of pneumococci that would kill a control mouse. It has been found that

there are in this country four groups of pneumococci in a serological sense, a discovery confirmed by therapeutic results. It is possible to prepare curative anti-sera against two of the groups while the other two fail to call forth useful anti-bodies. In any given case of pneumonia the type of infecting organism is determined by isolating it from the sputum and performing the agglutination test with the individual sera of Types I and II, whereupon should one of these react the appropriate anti-serum can be injected. The organisms are obtained for the agglutination test by injecting the sputum into a mouse's peritoneum, killing the animal after six to eight hours and using the rich growth of cocci in the peritoneal fluid as the bacterial suspension. With Type I the therapeutic results are very promising; with II helpful at times. Type III is the *Pneumococcus mucosus* yielding no useful anti-serum, while Type IV is a heterogeneous group possessing no serological uniformity and unable to call forth any valuable antibody in the injected horse. The serum is given intravenously in dose of 50-100 c.c. and repeated when the temperature rises again, sometimes every eight hours.

Meningitis Anti-serum.—Epidemic meningitis, caused by the *Micrococcus meningitidis intracellularis* or meningococcus, can be treated by anti-serum. The cocci owe their power to endotoxins and pus formation, exerted chiefly in the coverings of the central nervous system. They are present in the spinal fluid, only being found in the blood early in an ordinary attack or in highly septicemic cases. Anti-serum is made by injecting horses with first dead then living bacteria until its serum shall have acquired high agglutinative, opsonic and bactericidal power. The horse's serum is separated, preserved as usual, and used by injecting into the arachnoid space by lumbar or cranial puncture. This route is selected to bring the anti-serum into close relation with the cocci since by the subcutaneous or intravenous routes insufficient anti-bodies pass from the blood to the meningeal fluid. It should be given also into the blood stream

in order to prevent septicemia. Its effect is to increase phagocytosis and cause bacteriolysis. No satisfactory standard has been devised. Dosage varies but a safe guide is to inject under the meninges 75 percent as much as the fluid removed at lumbar puncture. Intravenous dosage should be 20-50 c.c. The anti-serum probably has no prophylactic value.

Anti-plague Serum.—Yersin, a French bacteriologist, treated horses with living cultures of plague bacilli, and after a long period of immunization used a serum which either effectually vaccinated an individual against the plague, or greatly modified the disease after it had once begun.

The action of the serum is bactericidal, as well as anti-toxic. The dose varies with the stage of the disease; 20 c.c. is an effective prophylactic dose, while from 20 to 300 c.c. have been used often as curative doses.

VACCINATION

By the use of attenuated, or killed microorganisms, it is possible to effectively vaccinate men and animals against many diseases, notably, small-pox, hydrophobia, plague, cholera, typhoid fever, anthrax and quarter-evil.

Any of the bacterial products used as prophylactics are sometimes called vaccines, the word being borrowed from small-pox vaccine. It is better to use the word *bacterin* for the purpose, even when they are given prophylactically. *Bacterin* is employed for the dead bacterial masses used therapeutically. *Sensitized bacterins* are vaccines that have been exposed to the action of the respective anti-sera before their use. It is the purpose of this procedure to prepare the organisms by combining them with the homologous anti-bodies so that when injected they have only to combine with complement to begin their immunizing effect. Theoretically this is correct but the practical value has not been fully confirmed.

Vaccination Against Small-pox

Jenner's observations in 1794 established the etiological relationship between human variola and cow-pox. Human small-pox virus passed through a cow will lose its power to produce typical variola but will produce in man a local condition called vaccinia which, upon recovery, leaves behind immunity to variola.

By the term vaccination, in its strict sense, we mean the application of attenuated small-pox virus, weakened by passage through kine, to human beings and infecting them with the modified disease. The disease is localized at first at the site of inoculation, and a bleb or vesicle forms. As a rule the disease does not become generalized. It creates, in the vaccinated individual, an active immunity against small-pox. The toxins diffused through the blood-stream stimulate the cells of the body into forming either anti-toxic or other anti-bodies. These various substances, as yet unknown, remain for a long period within the body of the vaccinated person and may protect it for years against invasion and infection with the cytorcytes in virulent form. A person who has variola cannot be vaccinated, subsequently he is immunized against vaccinia by this attack of variola, just as he can be immunized against variola by vaccinia infection.

Since Jenner first discovered that cow-pox introduced into the body prevented small-pox, it has been the world-wide custom to use either the dried virus or liquid glycerinized virus from the cow or human beings in the process of vaccination. It has been found that human virus generally used was likely in rare instances to transmit syphilis, so it is now the universal custom to use cow virus. This virus is collected from fresh vesicles in calves or young heifers, as clean as possible, as it is used as seed to inoculate the animals and the operation is done under strict antiseptic precaution. After a week the virus is collected under similar antiseptic precautions by scraping the base of the vesicle with a

sterile curette. The pulpy substance thus obtained is mixed with glycerine and stored for a month or more. The action of the glycerine is to rid the virus of many of the bacteria, through, it is supposed, a hydrolytic action. This virus is then rubbed into the skin of the individual to be vaccinated under strict aseptic precautions. At the end of a week, a pearly white vesicle is formed, and it is then considered that vaccination has "taken" and that the individual is protected against variola. This action of immunization is supposed to be complete on the fourth day after the virus has been introduced. This is a matter that is difficult to decide, but the immunization process is, no doubt, a very slow one, like every other immunizing process where the immunity is autogenous and active, and not passive, as in the case of diphtheria anti-toxin.

Persons who are entirely immune to variola will show twenty-four hours after vaccination a distinct red areola without vesicles or pustules; this is the reaction of immunity. Recent successful vaccination leaves the patient in a condition of resistance and four to six days after revaccination an areola surmounted by a reddish papule is seen; this is vaccinoid.

Vaccination Against Cholera

By the attenuations of cholera spirilla, Haffkine has produced vaccines which effectively protect individuals against infection with cholera, or if they become infected with the disease, it is so modified that they can, and do, more easily recover. He employs two vaccines, a weak one and a stronger one. The weak one is used to prepare for the stronger one, which is the effective vaccine.

The weak, or first virus, is prepared by growing the cholera vibrios at a high temperature, $39^{\circ}\text{C}.$, in a current of air. The stronger is prepared by passing the vibrios through a series of guinea pigs, so increasing the virulence that the virus is invariably fatal to the guinea pigs in eight hours. After cultivating this

virus on agar, the surface growth is washed off with sterile water (8 c.c.) and $\frac{1}{8}$ part of this is used as a dose. As the virus rapidly attenuates it must be reactivated by passing it through guinea pigs from time to time.

The first injection is given in the flank, and the second follows in five days. Accordingly as the symptoms are severe, so will the resulting protection be strong. Haffkine has given 70,000 injections without an accident. The following results were obtained by Haffkine who worked in India for the British Government:

Population	Case—Cholera		Deaths	
	Total	Percent	Total	Percent
Non-inoculated, 1,735.....	171	10.63	11.3	6.51
Inoculated, 500.....	21	4.20	19.0	3.80

The immunity conferred by this mode of vaccination is not complete until ten days after treatment. It is possible to vaccinate with these relatively virulent bacteria because they are given under the skin, a place where life of the vibrios soon ceases.

During an attack of cholera the vibrios do not enter the blood but remain in the deep layers of the intestinal mucosa.

Vaccination Against Typhoid

By the injection of sterilized cultures of typhoid bacilli, it is possible to create an immunity of a moderate kind against enteric fever. The method was perfected by Wright, and his mode of procedure is to secure a culture of typhoid both virulent and able to call forth a large amount of anti-body in the injected person, which is tested on guinea pigs, and the minimum lethal dose for a 100-gram guinea pig is used as the dose for man. This dose varies from .5 c.c. to 1.5 c.c. of a culture sterilized by heat

at 60°C., and preserved with lysol. After the injection there is often redness and pain at the site of inoculation, some fever and lymphangitis. The method at present in use in the United States is to employ a twenty-four hour agar slant or bouillon culture, killed by heating as above; these are suspended in saline with trikresol or phenol and counted in a hemocytometer. In order to control the toxicity of this suspension a mouse is injected subcutaneously with a billion; it should live at least five days. The doses are 500 million, 1,000 million and 1,000 million eight to ten days apart.

The results of Col. F. F. Russell, U. S. A., a man who has had much experience, since he was in charge of the army vaccinations, are interesting and instructive. He says:

1. "Anti-typhoid vaccinations in healthy persons is a harmless procedure.
2. It confers almost absolute immunity against infection.
3. It is the principal cause of the immunity of our troops against typhoid in the recent Texas maneuvers.
4. The duration of the immunity is not yet determined, but is assuredly two and one-half years and probably longer.
5. Only in exceptional cases does its administration cause an appreciable degree of personal discomfort.
6. It apparently protects against the chronic bacillus carriers, and is at present the only means by which a person can be protected against typhoid under all conditions.
7. All persons whose profession or duty involves contact with the sick should be immunized.
8. The general vaccination of an entire community is feasible and could be done without interfering with general sanitary improvements and should be urged wherever the typhoid rate is high."

The wisdom of these conclusions has been abundantly proven in our army during the World War when practically no typhoid fever occurred in vaccinated men. It might be added that no

immunizing procedure is perfect and this one does not justify the drinking of water known to be polluted.

Vaccination against all the typhoid fevers, due to the typhoid bacillus, the paratyphoid bacillus A and B, can be accomplished by combining these organisms in one vaccine. The form now in common use, as employed by our armed forces, is one suspension in each cubic centimeter of which are contained 1,000 million typhoid bacillus, 750 million of paratyphoid A and 750 million of paratyphoid B. Three doses, .5 c.c., 1 c.c. and 1 c.c. are given ten days apart. This makes a total of 6,250 million organisms injected.

Vaccination Against Pneumococcus Infections

Experiences in South Africa by Lister and in the American Army by Cecil seem to hold out encouragement for the prophylaxis of pneumonia by vaccines of the respective coccus. The types of pneumococci prevalent in a district must be determined and used in the suspension. The preparations employed by Cecil contained the three fixed American types and were given in doses of 6,000 million four times at six to eight day intervals. Under war conditions some advantage was observed but available data are too few to form a general evaluation. Experimentation and human experience indicate the harmlessness of this procedure and use is recommended. The action of the vaccine seems to be stimulation of opsonins and of bacteriolysins.

Vaccination Against Diphtheria

According to the experiments of Behring, Theobald Smith and W. H. Park it is perfectly feasible and without danger to immunize children against diphtheria. As already mentioned immunization of horses is begun with nearly neutral toxin-anti-toxin mixtures. For immunization of children only such are used. Solutions of the toxin and anti-toxin are made to contain 1 unit

of the latter and 60 percent and 80 percent of a unit of the former. Two or three injections are given, the first or the first and second of the former strength, the last having the higher value of toxin. A local reaction occurs and not infrequently a general one—fever, malaise. Immunity requires a week to start and about a month to be fully developed. It is believed to last at least four years. Immunization need only be given to those who have a positive Schick test.

Vaccination Against Plague

Haffkine, in India, has vaccinated natives and others against plague by somewhat the same methods employed in anti-cholera vaccination. The *B. pestis* is cultivated in flasks of bouillon; as it grows, the stalactite-like scum on top is shaken from time to time to the bottom of the flask. After growing for six weeks in the bouillon, the culture is killed at 70°C. for three hours. It is then used as vaccine, 3 c.c. is the usual dose for man, 2 c.c. for woman, and children still less. After the inoculation, heat and redness appear at the site of inoculation, and the patient feels ill and has some fever. Haffkine holds that immunity against the plague is complete in twenty-four hours after vaccination. His results are at times really very good.

The Indian Plague Commission reported that the measure was valuable as a means of preventing infection; while it was not an absolutely certain means, yet it sensibly diminished the death rate. The immunity lasts about a month. Such vaccines are not to be used after attack has started.

Vaccination Against Anthrax

Of all forms of vaccination against disease with attenuated bacteria this is the most successful. Its use is confined to domestic animals, sheep, cattle, and horses, and has reduced the mortality in the country where it is used from 10 percent to .5

percent. The method requires the employment of two vaccines made of attenuated anthrax bacilli. No. 1 is a culture of bacilli attenuated by growing them at a high temperature, 42.5°C ., in a current of air for twenty-four days. No. 2 is grown at the same temperature for only twelve days. The first vaccine is used to immunize the animal against the second, which causes a marked local reaction, and which is the real immunization agent against infection with virulent anthrax bacilli. The injections are given about one week apart. Many State Governments as well as the Federal Government of the United States supply the vaccine gratis to stock raisers and others.

Vaccination Against Black-leg or Quarter-evil

Quarter-evil, or Rauschbrand, is due to a specific bacillus. Vaccination against this disease may be accomplished by inoculating with a powder consisting of dried muscle from the affected part of infected animal. There are two vaccines, No. 1, and No. 2. The first is prepared by heating (and thus attenuating) the bacilli up to 103°C . The second is prepared by raising the temperature up to 93°C . These vaccines are given at a short time apart, and the immunity is effective. The method is valuable to stockmen.

Vaccination Against Tuberculosis

There is at present no safe and satisfactory prophylactic measure for the production of increased resistance in man to tuberculosis. In cattle the repeated injection of bacilli of the human type has been found capable of raising the animal's resisting power to a sort of immunity. The therapeutic use of the various tubercules has on the other hand, met with better success, and it seems that for human medicine at least that they have won a place in the treatment of surgical tuberculosis. They may be also of value in pulmonary disease.

The Tuberculins

The toxin of the tubercle bacilli (old tuberculin) is prepared by growing the organism for a long period in glycerinized veal broth, after which the flasks are steamed in a sterilizer for an hour or more, and then the bacilli are filtered out through porcelain filters. The filtrate is reduced by boiling to one-tenth of its bulk, and to this .5 percent of carbolic acid is added as a preservative. If this toxin, even in minute doses, is injected under the skin of a tuberculous animal, it acts as a powerful poison. In a few hours, it causes a rapid rise of body temperature, accompanied by nausea and, perhaps, vomiting. About the localized foci of tuberculosis, a vigorous reaction occurs. Around indolent old sores and other lesions there is a tendency to heal by the casting off of necrosed tissues, and the infiltration of the perituberculous area with leucocytes. In lupus (tuberculosis of the skin) redness and heat occur about the lesion. This febrile phenomenon following the injection of tuberculin into tuberculous animals is a valuable diagnostic feature toward the recognition of tuberculosis in animals and in man. In 90 percent of cases the reaction is trustworthy.

Its use in man has been much questioned, as it is thought by some to disseminate the disease from original and confined foci. This, however, has been denied. Many able clinicians use it and recommend it (Osler, Trudeau, Musser).

Koch's new, or T.R. tuberculin was, like the old, designed by him as a therapeutic agent for the cure of tuberculosis. It is made by pulverizing the bodies of living tubercle bacilli and dissolving the residuum in an indifferent fluid, centrifuging this and collecting the sediment which is Tuberculin Rest, T.R. The solution above this sediment containing soluble substances from the bacillary bodies is Tuberculin Obers, T.O. It produces a more intense reaction than the old tuberculin. Like the old, it is used in the treatment of lung, bone, laryngeal, and skin tuber-

culosis. It certainly causes a local reaction about tuberculous foci, and no doubt aids in the building up of healthy tissue.

The dose of tuberculin for testing purposes should be .5–2. mg. for a child, 2.–6. mg. for a young or weak person, and 5.–10. mg. for a larger person. It is well to give the highest dose that it is believed the patient will stand in order to get a prompt and definite result thus avoiding the necessity of a repetition. Repeating the injection of such amounts is not without danger as it might light up a latent lesion. Any focus that is at the bottom of a clinical condition requiring such a test, will give a positive reaction with the quantities mentioned. For therapeutic purposes one begins with an injection of .0000001 gram or smaller and increases slowly according to the patient's condition. Tuberculin should only be administered by experts.

Mallein

Mallein is a preparation made from the toxin of the glanders bacilli, and is prepared precisely as the old tuberculin. By increasing the virulence of the glanders bacilli, by passage through a series of guinea pigs, a highly virulent bacillus is obtained. It is then grown in glycerinized bouillon for a month at 37°C. The resulting fluid is sterilized by heat and filtered through a Pasteur filter. The filtrate is evaporated to one-tenth its quantity when intended for conjunctival use or left in its natural state when for subcutaneous use. A small amount of carbolic acid is added in order to preserve it.

In a horse with glanders, the injection of mallein is followed by a large painful swelling at the injection site. With this there is a rise of temperature, which is the diagnostic reaction that indicates infection with glanders. In this respect the reaction is like tuberculin. In healthy horses no rise of temperature follows the injection, and the resulting swelling more quickly subsides. A convenient test is the introduction of a drop of concentrated mallein into the conjunctival sac. Positive reaction is

indicated by lacrimation and purulent collections within twenty-four hours. Mallein has been used as a prophylactic agent against glanders with some success, and treatment can be carried on with it in valuable animals.

Immunization Against Hydrophobia

While the actual causal agent of hydrophobia has thus far eluded bacteriologists, certain well-marked histologic lesions have been discovered in the ganglia of the central nervous system, and in the medulla, which are not found in any other disease. This dispels all doubt as to the fact that hydrophobia is a real clinical entity.

It is possible to immunize animals and man against this disease by the use of attenuated virus. In common with many other viruses, that of hydrophobia can be weakened through the action of either heat, drying, light, or chemicals. Pasteur found that by drying the spinal cords of rabid animals for two weeks, they become totally avirulent. If the cord is dried but three or four days, the virulence is but slightly reduced. Immunity to rabies can be produced by injecting minute quantities of the poison, and then gradually increasing the dose until virulent virus can be employed.

Recent work seems to indicate that simple dilutions of the virus so that minute quantities are used, can be employed prophylactically instead of dried material.

Modification of the amount of poison used may be affected by employing equal quantities of spinal cords from rabid animals that have dried varying lengths of time. The vaccine consists of pieces of cord, 1 cm. in length, from rabbits that have been killed by inoculation with fixed virus. This is emulsified with sterile salt solution. Cord that has dried for fourteen days is first injected, after which cords that have dried fewer and fewer days, until, finally, one that has dried only three days is injected.

In cases of bites by rabid dogs on the face or head, the vaccination must be rapid, so two injections per diem are given. In

Berlin the weakest injection used (the first) is made from a cord that has dried but eight days, and the course is much quicker. It was at first thought that short drying might carry over too much virus but in order to treat certain serious head bites, cords of 3 and 4 days drying were tried not only without damage but with promising results. Now in threatening cases treatment may be begun with 3 day cords, then 2 day cords. The effect of this mode of inoculation is to produce in the bitten individual a very rapid active immunity, quicker in its action than the infection. The treatment is solely prophylactic and in no way curative. If symptoms of rabies have set in, the treatment is of no avail. In rabies the incubation period is about six weeks, so that there is plenty of time to immunize the patient by injection with attenuated virus.

Since the immunizing process is always begun *after* the bite of a rabid, or supposedly rabid dog, it differs from other vaccinations, which are resorted to *before* infection.

Results of Treatment.—Among those bitten by rabid animals the total mortality before the introduction of vaccination was not less than 10 percent. Among the same class of patients in the Pasteur institutes, the death rate of all cases, early and late, has been reduced to a fraction of 1 percent. Those cases in which the bites are on the head, are always more serious, and the mortality is higher. Like tetanus the virus travels, it is supposed, from the site of injury to the central nervous system by way of the nerves. If the bite was on the toe, it would take longer for infection to reach the brain, than if it was on the upper lip. This is a very plausible explanation of the varying incubation periods in both tetanus and hydrophobia.

Coley's Fluid in the Treatment of Tumors

This method of treatment is in no wise a prophylactic one, but strictly a curative one. It consists in the injection of the toxins of streptococci, in the hope that they will cause a shrinking, or dis-

appearance of malignant sarcomata. An attack of erysipelas (it has long been observed) occurring in a patient with some malignant disease, has the effect of causing a disappearance, or retrogression, of the tumors. Artificial infection with streptococci was then practiced with the idea that it might produce the same effect. But this was found to be dangerous. Coley prepared toxins of streptococci by allowing them to grow with the *B. Prodigiosus*. The mixture after a long period of incubation was sterilized by heat, and the fluid thus obtained was injected into the tissues. Virulent strains of streptococci are used and the dose of the dead culture is about half a drop given under strict anti-septic precautions. The best results are obtained in spindle-cell sarcoma, and the poorest in the melanotic variety. The method by no means should be employed where the tumor can be removed by operation. It cannot supplant the knife, and only in inoperable cases or as a supplementary treatment where other forms of treatment are employed, should it be used.

Opsonins and Opsonic Index

Peculiar substances in blood serum have been called by Wright and Douglass *opsonins* (Greek: prepare food for). If fresh blood is mixed with an emulsion of some bacteria and then incubated for half an hour, it will then be found that many of the bacteria are within the polymorphonuclear leucocytes. If the serum is washed away from the leucocytes before adding bacteria, none of the latter will be found within the leucocytes. This proves that the serum has some influence on phagocytosis. In order to show that this effect is on the bacteria rather than on the leucocytes, the bacterial suspension may be treated with some serum for half an hour and then washed free from this serum by means of a salt solution in a centrifuge, and then mixed with some serum-free leucocytes; then it will be found that phagocytosis occurs as before. The bacteria have been "sensitized." According to Wright this action is comparable to cooking or peptonizing.

Phagocytosis then depends upon the action of serum upon bacteria, which are coped with in the body, first by the action of the serum, and then by the leucocytes. It is thermostabile.

The quantitative action of phagocytosis may be estimated by Leishman's method. He mixed blood and an emulsion of bacteria in salt solution in equal

quantities, and allowed them to stand for thirty minutes in the incubator. After this the mixture was stained and the average number of bacteria per leucocyte was obtained. The result was known as the *phagocytic index*.

Wright has devised the following technique. Young cultures, a few hours old, are employed. These are scraped off agar tubes and mixed with salt solution. After this has sedimented, the supernatant fluid is separated from the bacterial masses by a centrifuge; is pipetted off, and preserved.

Washed leucocytes are obtained by collecting 2 c.c. of blood in 30 c.c. of salt solution containing 1 percent citrate of soda to prevent blood coagulation. The serum and citrate of soda are separated from corpuscles by washing twice in a centrifuge. The upper layer of the sediment is rich in washed leucocytes, and is used in the experiments.

To obtain the opsonic index, blood serum from various cases is collected. In the case of staphylococcus infection—say furuncle—the blood serum is drawn from the patient and, with equal portions of an emulsion of staphylococci (young culture), and a suspension of washed corpuscles, is thoroughly mixed in a pipette, which after the ends are sealed, is placed in an incubator for fifteen minutes. A drop of the mixture is then spread upon a slide; fixed, and stained with Jenner's stain. The number of staphylococci in 50 polynuclear leucocytes is determined and divided by 50 to obtain the average.

At the same time that this experiment is being performed, some normal serum should be used in another experiment; an emulsion of staphylococci and washed leucocytes being used as above. After pursuing the same steps in this experiment as in the first, the average number of staphylococci per leucocyte is determined.

To obtain the *opsonic index*, it is necessary to know the ratio of staphylococci in the leucocytes treated with furuncular serum and with normal serum. If the normal serum leucocytes contained 10 staphylococci, and the furuncular serum contained 15, the index would be 1.5.

In the case of tubercle bacilli, the latter must be heated to 100°C. to kill them, otherwise they will be agglutinated by the serum, and a homogeneous emulsion will not be obtained. After heating, the clumps must be broken up by grinding the masses in an agate mortar, adding a little salt solution from time to time until the mass is thoroughly broken up. The bacilli must then, after phagocytosis, be stained by carbol fuchsin and decolorized with acid alcohol. If the leucocytes are left too long in contact with the organisms they may become so engorged as to prevent counting, the number increasing from 5.7 percent after five minutes to 28.5 percent in two hours.

Highly immunized anti-bacterial serums have much greater opsonic powers than have normal ones, anti-streptococcus and anti-pneumococcus sera being especially powerful toward streptococci and pneumococci. It is pos-

sible to increase the opsonic powers of the blood of an individual suffering from an infection, by vaccinating him with killed cultures of the organism with which he was infected.

The determination of the opsonic index is a long and tedious affair so that in practice it is only used when it is necessary to estimate the value of the vaccine treatment or to control dosage. Under ordinary circumstances clinical phenomena will indicate the correctness of dosage and interval but certain cases that fail to do well should be checked by opsonin indicators. The value rises slowly and steadily with appropriate vaccine, dosage and interval, falls with too large quantity, shows no change with inadequate quantities.

The Local Reactions or Tests.—We have learned in the past few years that the skin and mucous membranes will react more or less specifically to the bacterial proteins. It is a form of allergie (see page 66). There have been developed local tests for tuberculosis, syphilis, typhoid, glanders and other diseases. The first two being the most important are considered below. The others are of similar nature.

Tuberculosis.—If tuberculin of any form be rubbed into an abraded skin area (Von Pirquet's cutaneous) or injected between the layers (Moro's percutaneous) of the skin a red maculopapule or even vesicle upon an inflamed base will appear within twenty-four hours. There may be a mild general reaction of fever and malaise. A positive reaction to such an installation simply indicates the presence of a tuberculous lesion and that an allergic state of the skin exists but does not show whether or not the lesion is active. For this reason it is only of value in children since three-fourths of adults are believed to have a healed lesion within them. Not only upon the skin but upon the conjunctiva can this reaction be obtained.

Syphilis.—The poison of the *Treponema pallidum* is called luetin. It is made by grinding up in salt solution a culture of the germ, heating the resulting mass to 60°C. for an hour and preserving it with phenol. If this be instilled into an abraded skin area a maculopapule or nodular eruption occurs in a syphilitic. This positive outcome, however, appears only in late cases, those of tertiary stages and in treated cases. It therefore complements the Wassermann reaction, being positive where this is apt to fail.

Schick Test.—It has been found by Schick and others that if $\frac{1}{50}$ minimum lethal dose of diphtheria toxin in .2 c.c. of saline be injected into the skin of a person, a swollen, pink, tender area will appear in persons susceptible to infection with Klebs-Löffler bacilli. If no such reaction occurs, the person is not susceptible. This depends upon the fact that if anti-toxin be present in the blood it will combine with the toxin and no reaction will appear, while if no anti-toxin be present the toxin is free to exert its effect. It has been

determined that persons giving a negative reaction need not receive immunizing doses of anti-toxin. About 80 percent of adults and 30 to 40 percent of children are immune. A negative reaction seems to indicate that the blood contains $\frac{1}{30}$ c.c. more unit of anti-toxin per cubic centimeter.

Isoagglutinin and Isohemolysin

There are frequently in the blood of animals, lytic and agglutinative anti-bodies for the red cells of other members of the same species. By this is meant that if the blood of a man be mixed with that of another the cells of one of them may be clumped or dissolved. This is of considerable importance to the surgeon who wishes to transfuse blood, for were he to use a donor whose blood was unlike that of the recipient the latter would have a serious chill, indications of blood destruction and shock, and he might die.

These two substances are probably independent in action but are so commonly found acting in harmony that they may be considered inter-dependent. Landsteiner and Jansky divided persons into four groups according to their agglutinin and agglutinogen. Moss made the same observation but classified them differently.

JANSKY'S GROUPING

Cells	Serum				Percent
	I	II	III	IV	
I	—	—	—	—	42.84
II	+	—	+	—	10.36
III	+	+	—	—	41.38
IV	+	+	+	—	5.42

This means that the cells of Type I are not agglutinated by any of the serums, whereas the cells of Type IV are clumped by all serums but their own.

Agglutination is accomplished according to Landsteiner by two agglutinins a and b which are both contained in serums clumping all type of corpuscles, whereas both are absent in serums clumping no cells. Agglutinable bodies, represented by a and b, are supposed to be present in the respective cells which can be clumped, absent in the others. Group I therefore contains agglutinins A and B but does not contain agglutinable bodies a and b. Other groups

would be explained on the same basis. While Jansky's methods have priority in time of publication the American surgeon has during the great war become accustomed to the Moss grouping which is as follows:

Cells	Serum			
	I	II	III	IV
I	—	+	+	+
II	—	—	+	+
III	—	+	—	+
IV	—	—	—	—

It will be seen that this is merely the reverse of the first one and that Type IV has agglutinins but no agglutinable bodies.

Agglutinins and agglutinable bodies may be absorbed from a given blood by saturation with their respective antigen.

In transfusion, homologous bloods should be mixed, but groups I or IV respectively may be considered as universal donors since experience shows it safe to use a blood which agglutinates the recipient's cells. It is however, incorrect to use a blood whose corpuscles are clumped by the recipient's serum. The agglutinative titer of human blood is not very high, 1-10 to 1-25, and as the entering serum is diluted the agglutination titer would be exceeded.

Further experiments on the independence of the two anti-bodies would indicate that usually agglutination precedes hemolysis but this need not be so, in a small percentage of cases. For practical purposes agglutination tests are sufficient indications of the compatibility of bloods. Tests for compatibility take the form of (1) direct mixture of the blood of recipient and of donor and (2) the testing of donor's blood by standard serums.

1. Patient's blood A—serum separated from the clot. B—cells from defibrinated blood washed in saline and resuspended in saline. Donor's blood a—serum separated from the clot, b—cells from defibrinated blood washed in saline and resuspended in saline.

Test A—4 parts b 1 pt.

a—4 parts B 1 pt.

Incubate at 37° for one hour.

If bloods be compatible no change will occur in the blood cells. If donor's corpuscles be agglutinated by recipient's serum there will be clumping in the first tube; if the reverse, a change will occur in the second tube. This method while often used is more trouble and no more reliable than the following:

2. It is necessary to have for the group determinations, known sera of the various types, but for practical purposes types II and III will reveal any type corpuscles suspended in them. The blood to be tested is caught from a prick in the finger into a few drops of sodium citrate solution. One drop of this cell suspension is mixed with one drop of type II and of type III sera separately on a slide. After a period of five to thirty minutes clumping may occur. If it occur in both, the blood is by the Moss scale group I; if in neither it is group IV; if clumping occur in serum III and not in serum II, the blood is group II; if clumping occur in group II and not in group III the blood is group III.

CHAPTER V

STUDY OF BACTERIA

Bacteria are studied in the following various ways:

1. Morphological characteristics, form, size, motility, presence of spores, granules, capsules, and flagella. Reaction of protoplasm to dyes and reagents.
2. Characteristics of growth in culture media; appearances of culture; chemical activities; production of acid, gases, toxins, colors, etc.; reactions to heat, disinfectants, light, etc.
3. Study of the action of bacteria on the tissues of man and animals, and of the toxins on the tissues and functions of the various organisms.

The simplest way to study bacteria is to make a hanging drop of a fluid containing bacteria, and observing the organisms under a microscope. To do this, a cover-slip, and a slide with a concavity ground in it are used. A drop of bacteria laden fluid is placed on the cover-glass, and after the edges have been smeared with vaseline, the cover-slip is inverted over the concavity in the slide, and the bacteria can then be examined with either the dry $\frac{1}{6}$ inch, or the one-twelfth oil immersion objective. If the preparation is kept warm for some time, various vital phenomena may be noted. Direct division, sporulation, motility, agglutination, and bacteriolysis can be studied by this means. Instead of using a fluid, a block of nutrient agar may be cemented to the cover-glass; after the bacteria have been planted on the agar, the various vital phenomena may be noted.

All minute bodies, whether they be bacteria, dust particles or granules of india ink in suspension, exhibit a trembling vibrating motion called the Brownian motion. Motile bacteria either move

so swiftly that the eye can hardly follow them, or they may merely roll or wiggle across the field slowly. Direct division, if proceeding under the best conditions, requires but fifteen to forty minutes. It is best observed in a warm stage or when working in a room kept at a temperature of 35°C . Sporulation occurs differently in different species. In some it will be found soon after the culture has been removed from the incubator, while in others several hours are required. Sporulation, it must be remembered is a resistant stage when unfavorable conditions are met.

The Gruber-Widal reaction is thus studied. A drop of the serum and bullion culture, mixed in proper proportions, is dropped on a cover-slip, which is then placed, drop downward, over the cavity of the slide (*hanging drop*, Fig. 20) (see Agglutination).



FIG. 20.—Hanging drop, over hollow ground slide. (Williams.)

Staining bacteria is a matter that is easily accomplished, and very many staining solutions and methods have been invented for this purpose.

The simplest procedure is to take a drop of pus, blood or culture, and spread it upon a very clean slide with a sterilized platinum needle. The matter must be spread thinly and evenly. After the water has evaporated and the preparation has become dry without the use of heat, it must be fixed. To do this various agents are used. The object of the fixing is to coagulate the protoplasm of the cells, and to fasten all the smeared matter fast to the glass, so that the staining fluid and water will not wash them off. This is accomplished, for bacteria usually, by holding the smeared slide in the apex of a bunsen flame until quite warm to the hand. Great care must be used not to char the film. Experience is needed to fix slide smears correctly. The beginner would do well to use cover-slips. If a cover-slip is used it must be passed through the flame three times rapidly. After fixing and thorough cooling,

the staining fluid is poured on, and after remaining a few minutes is poured off and the slide is washed, dried by blotting paper, and examined. If a cover-slip has been used a drop of balsam is put upon a clean slide and the cover, smeared with stained bacteria, is inverted on the balsam. Upon the stained bacteria themselves (if a cover-glass has not been used) or upon the cover-slip a drop of cedar oil may be placed, and the preparation examined with a one-twelfth objective. This is one of the simplest staining procedures practised in bacteriology. Other more complicated methods will now be described.

Besides heat, absolute alcohol, methyl alcohol, or formalin may be used as fixatives. Some stains are made up with methyl alcohol, and instead of fixing by heat, the stain is merely dropped upon the dried film, and the bacteria are fixed and stained by the same solution at the same time, water being added for differentiation at the end.

Aniline dyes are almost entirely used as stains in bacteriology and these are divided into two classes, the basic and acid stains, according as their staining properties depend upon the basic or acid part of the molecule. Basic dyes stain nuclear tissues of cells and bacteria. The acid are used as contrast stains and do not color bacteria, but tissues in which they may be imbedded.

The common basic stains are methyl violet, and gentian violet, methyl green, methyl blue, and methylene blue, thionin blue, Bismarck brown, fuchsin, and saffranin. These are used for staining different bacteria under different conditions. The most useful stain is methylene blue, since it is difficult to overstain with it, and it is very easily applied. It has been found that certain physical and chemical conditions are necessary for successful staining with aniline dyes. Alcoholic solution of dyes entirely devoid of water do not stain; absolute alcohol does not decolorize bacteria after staining with aniline colors, while diluted alcohol decolorizes readily. The more completely a dye is dissolved, the weaker is its staining power. A dyestuff unites, as a whole, with the bacterial

plasm, forming, as it were, a double salt between the two. Certain substances, alkalies, carbolic acid, iron and copper sulphate, tannic acid, alum, and aniline oil, are added to a solution of aniline dyes, and they act as mordants, or fixatives, making the dye bite into the protoplasm of the bacterial cells. Spores, capsules, and flagella, are hard to stain, and special heavily mordanted stains are used to demonstrate them. Chemical reaction occurring in the cell protoplasm is of great value in differentiating bacteria. The presence of granules in bacterial cells is often only shown by the use of special stains, which deeply color them. Bacteria of the tubercle group are called "acid-fast," because, after being stained, it is difficult to decolorize them with acid solutions. These bacteria are hard to stain and resist decolorizing agents after they are stained.

1. Löffler's alkaline methylene blue solution consists of

Saturated alcoholic solution of methylene blue.....	30 c.c.
$\frac{1}{10,000}$ solution caustic soda solution in water.....	100 c.c.
Mix.	

This is the most useful of all the staining mixtures employed.

2. Zeihl's solution carbol-fuchsin consists of

Fuchsin.....	1 gram.
Carbolic acid crystals.....	5 grams.
Dissolved in 100 c.c. of water, to which is added 10 c.c. of absolute alcohol.	

This can also be made by taking a 5 percent solution of carbolic acid in water and adding sufficient saturated solution of fuchsin in water until a bronze scum persists upon the top. This is used for staining tubercle bacilli in sputum and sections. It must be heated when used for rapid staining. Tubercle bacilli can be stained in cold solution, if immersed over night in it.

3. Fuchsin solution.

Saturated alcoholic solution of basic fuchsin.....	1 c.c.
Water.....	100 c.c.

4. Bismarck brown solution.

Water.....	100 c.c.
Bismarck brown sufficient to saturate.	
Filter and use a contrast stain.	

5. Weigert's aniline gentian violet stain.

Gentian violet.....	1 gram.
Dissolve in absolute alcohol.....	15 c.c.
Distilled water.....	80 c.c.
Then add to this	
Aniline oil.....	3 c.c.
Mix, shake and filter.	
This stain can also be prepared by taking a	
Sat. watery solution of aniline oil.....	100 c.c.
Filter, then add	
Sat. alcoholic solution gentian violet.....	10 c.c.

Sterling's permanent solution is made by mixing 2 c.c. of aniline oil with 10 c.c. of 95 percent alcohol; the mixture is shaken and 88 c.c. of distilled water added; 5 grams of gentian violet powdered in a mortar receives the above fluid, added slowly while grinding; filter. This solution while expensive to make, requires only a small quantity, stains rapidly and keeps well.

This is a very intense bacterial stain used for demonstrating bacteria by the Gram method.

Gram's method of staining.

A cover-glass is spread with a smear of bacteria, or pus to be examined. After air-drying it, and fixing it in the flame, the aniline gentian violet is poured on, allowed to stand for three minutes, then poured off and the preparation treated with

Iodine crystals.....	1 gram.
Potassium iodide.....	2 grams.
Water.....	100 c.c.

for two minutes. This renders the purplish preparation grayish in appearance. Alcohol is now poured upon the preparation repeatedly until the alcohol does not dissolve any more color. A contrast stain of Bismarck brown or dilute fuchsin or safranin is

now used. If the bacteria on examination remain a dark violet-blue they are then said to stain by Gram's method, or are "Gram-positive." If they are decolorized they take the contrast stain and are said not to stain by this method, and are "Gram-negative."

Many bacteria stain in this way, and many do not. Important bacteria often may be differentiated in this manner.

Examples of Gram's stain are as follows:

Gram-positive—*Bact. aerogenus capsulatus*, *Bact. anthracis*, *Bact. diphtheriæ*, *B. tetani*, *Bact. tuberculosis*, *Streptococcus pneumoniae*, *Staph. pyogenes*, *Strep. pyogenes*. Gram-negative—*B. coli*, *B. dysenteriae*, *Bact. influenzae*, *Bact. mallei*, *Bact. pestis*, *B. pyocyaneus*, *B. typhosus*, *Diplococcus intracellularis meningitidis*, *Micr. catarrhalis*, *Micr. gonorrhoeæ*, *Spirillum cholerae*.

Thionin Blue, or Carbol Thionin

This is a useful stain, prepared thus:

Thionin blue.....	1 gram.
Carbolic acid.....	2.5 grams.
Water.....	100 c.c.

Filter. Good for staining bacteria in tissues.

Special Stains

Wright's Stain.—This not only stains, but fixes. It has a wide range of usefulness in a bacteriological laboratory for the staining of blood, pus, malarial parasites, trypanosomes, as well as many bacteria, and is prepared as follows:

.5 percent solution of sodium bicarbonate.....	100 c.c.
Methylene blue.....	1 gram.

Mix and heat in sterilizer one hour at 100°C. Cool, filter, then mix $\frac{1}{10}$ percent yellowish eosin in water until the mixture loses its blue color and becomes purplish. Of the eosin solution add 500 c.c. to each 100 c.c. of the methylene blue mixture. Mix and collect the abundant precipitate which immediately forms on a filter. Dry this and dissolve in methyl alcohol in the proportion of 1 gram of powder to 600 c.c. of the alcohol. This is the staining fluid. Keep well stoppered. Fresh alcohol may be added for that which evaporates.

This complex stain represents a type of which Jenner's, Leishman's, and Romanowsky's are members. To use this stain, a blood or pus film is spread and air-dried. The stain is then run on the slip, or slide, for one minute. After this time slowly drop distilled water in quantity similar to that of stain used. This is when the true staining takes place. After three minutes wash in distilled water, dry and mount. Nuclei, malarial parasites, trypanosomes, and bacteria are stained blue; red cells are stained pinkish-orange, while the granules of the leucocytes are stained pink, lilac, or blue, depending upon their character.

Giemsa's Stain

This stain is used for demonstrating the organism of syphilis, trypanosomata, granules and the like, and is prepared as follows:

Azur II Eosin.....	3 grams.
Azur II.....	8 grams.
Glycerine C. P.....	250 c.c.
Methyl alcohol.....	250 c.c.

Bacteria are often covered with capsules that are difficult to stain, and special methods have been devised to demonstrate them.

1. Air-dry the specimen.
2. Harden and fix in absolute methyl alcohol.
3. Dilute stain with distilled water, using one drop of stain to each cubic centimeter of water.
4. Cover preparation with dilute stain fifteen minutes to three hours (longer period for spirochætes).
5. Wash in running water.
6. Blot and mount.

Capsule Staining

Welch's Method.

1. Cover-glass preparations are made in the usual manner, and over the film after fixing, glacial acetic acid is poured.
2. Without washing off the acid, aniline water gentian violet is poured on.

Change the stain four or five times to remove the acid. Stain four minutes, and wash with 2 percent NaCl solution, not water. This demonstrates the capsule very well.

His's Method.

"A."

1. Make cover-glass preparation mixing specimen with blood serum. Fix in flame.
2. Stain for a few seconds with a half concentrated water solution of gentian violet.
3. Wash in weak potassium carbonate solution for a few minutes.
4. Dry and mount.

"B."

2. Dry and fix.
3. Heat and pour on the following stain, steaming thirty seconds:
 - (a) Saturated alcoholic solution of gentian violet..... 5 c.c.
 - (b) Water..... 95 c.c.
4. Wash in a 20 percent solution cupric sulphate.
5. Dry and mount.

Spore Staining

Spores resist stains, and when stained are hard to decolorize.

1. Dry and fix in the usual way.
2. Flood cover-glass with hot carbol-fuchsin; heat until it steams; repeat this once or twice. This stains bacteria and spores.
3. Wash in water.
4. Decolorize with

Alcohol	2 parts.
1 percent acetic acid.....	1 part.

5. Wash.
6. Counterstain with methylene blue.
7. Wash, dry and mount.

By this method, which is a simple and satisfactory one, the spores are stained a brilliant red, while the body of the bacilli are stained blue.

Flagella Staining

To a beginner flagella staining is difficult; there have been many well-known methods devised. The simpler are as effective as the more complicated but do not always make as pretty preparations.

Flagella, being processes extending from the capsule, are, like the latter, hard to demonstrate. They are not stained by the common bacterial stains. In general a powerful stain mixed with a strong mordant must be employed. Some methods appear to be not so much a staining method in the ordinary sense but either a precipitating of the stain in the substance of the flagella or else a decomposition of silver salts in the flagella substance. To stain flagella, a young culture grown on agar must be employed; glycerine agar must never be used. A mass of the organism is gently mixed with a drop of distilled water until a uniform emulsion is made. A dozen cover-slips carefully washed and cleaned by alcohol are thoroughly flamed in order to remove the slightest trace of grease. The watery emulsion of bacteria is then spread over the cover-slips evenly and thinly. After they are dry the bacteria are fixed by holding them for a minute just above the apex of the flame with the fingers. The following methods may be pursued:

Pitfield's Method Modified by Muir.

Two solutions are necessary for this method.

A. Mordant.

10 percent watery solution tannic acid.....	10 c.c.
Corrosive sublimate saturated water solution.....	5 c.c.
Carbol-fuchsin solution.....	5 c.c.

This forms a dense precipitate which must be removed by the centrifuge, or sedimentation, and the clear fluid, or mordant, is stored in a bottle. It keeps for two weeks.

B. Stain.

Saturated watery solution of alum.....	10 c.c.
Saturated alcoholic solution gentian violet.....	2 c.c.

This keeps but two or three days.

Flood the cover-slip with the mordant and gently steam for one minute, then wash and dry thoroughly, pour the stain on and steam for one minute more. Wash, dry and mount.

This method yields very good results.

Pitfield's Method.

This is the simplest stain and the easiest to use, but does not give the good results that the previous one does. But one solution is needed, this is made in two parts and mixed.

- | | |
|--|---------|
| A. Tannic acid..... | 1 gram. |
| Water..... | 10 c.c. |
| B. Saturated watery solution alum (old)..... | 10 c.c. |
| Saturated alcoholic solution gentian violet..... | 1 c.c. |
| Mix. | |

A heavy precipitate is formed by this process which is useful in the staining. The stain is almost a saturated solution of alum and tannic acid, and when it becomes supersaturated by evaporation and heat, staining takes place. After this the process is very simple. The cover-slip is carefully flooded with the stain and warmed for a minute over the flame of a bunsen burner, turned very low, until steam arises. Not too much stain should be run over the cover-slip. After steaming occurs, the stain should remain for a minute, then the preparation is washed, dried and mounted. It will be found that the best stained flagella are on those bacteria nearest to the edges where the evaporation has been most intense. If the preparation is not equally stained, Weigert's aniline gentian violet can be run on for a minute to deepen the color.

Löffler's Method.

This is the original flagella stain and is a very good one.

It is made as follows:

- | | |
|---|---------|
| A. Mordant | |
| 20 percent watery solution tannic acid..... | 10 c.c. |
| Sat. solution ferrous sulphate..... | 5 c.c. |
| Fuchsin sat. alcoholic solution..... | 1 c.c. |
| Mix. | |

B. Stain

Carbol-fuchsin.

Proceed as in the previous methods.

The most important steps in flagella staining are to clean the cover-slips thoroughly, to mix the culture with water and have no culture media with it, to fix gently, and not to overheat the stain. Even in expert practised hands it is not always easy to demonstrate flagella readily.

Neisser's method of staining the diphtheria bacillus.
Two stains are needed (Fig. 21):

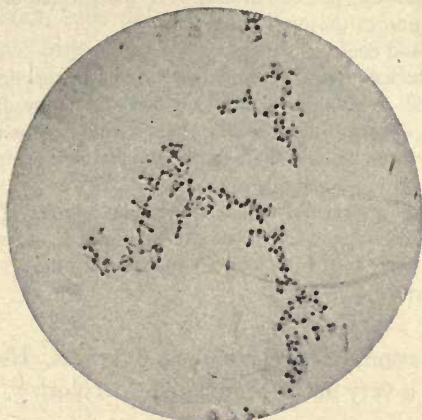


FIG. 21.—B. Diphtheria stained by Neisser's method. (Williams.)

A. Methylene blue.....	1 gram.
95 percent alcohol.....	20 c.c.
Water.....	950 c.c.
Mix and add	
Glacial acetic acid.....	50 c.c.
B. Vesuvín.....	2 grams.
Distilled water.....	1000 c.c.

The staining steps are as follows:

1. Prepare film, fix and dry.
2. Pour on "A" for thirty seconds.
3. Wash well in water.
4. Dry and pour on "B" for thirty seconds.
5. Wash, dry and mount.

The protoplasm of the bacilli will be stained brown, and the characteristic (diagnostic) chromatin points will be stained a deep blue black.

Tubercle Bacillus Stain

1. Spread the sputum, pus or culture, over the surface of the cover-slip. Allow the preparation to thoroughly dry.
2. Fix in flame and cool.
3. Pour carbol-fuchsin over the slide and heat with steaming for five minutes. Young bacilli in tubercles and other fluids are very difficult to stain in this way. The preparation containing them should be stood in cold carbol-fuchsin for twenty-four hours. This method stains everything on the slide.
4. Wash in water.
5. Decolorize the preparation with a 25 percent solution of sulphuric acid in water until the red color is lost. Repeat this once or twice.
6. Wash and counterstain with Löffler's methylene blue.
7. Dry and mount.

Gabbet's solution, methylene blue 2 grams, H_2SO_4 25 c.c., water 75 c.c., is a very useful, convenient decolorizer and counter-stain for sputum.

In such a preparation, if tubercle or other acid-fast bacilli are present, the bacilli will be colored a brilliant red, while the pus cells, epithelial cells, and other bacteria will be stained blue.

The microscope dark field illumination enables one to see flagella and capsules. This illumination is obtained by blocking out the central portion of the Abbe condenser in the substage of the microscope. Light is admitted only from the sides and objects in the field at the point of crossing of the rays reflect these from their sides. India ink may be used as a background for bacteria that stain poorly and have low refractive index.

Protozoa are stained by Wright's or Giemsa's method in one of its various forms. Spirochetes, particularly that of syphilis, may be stained by Giemsa's method but show up more clearly in the following technic of Stern.

1. Dry film in incubator for several hours.
2. Immerse in 10 percent aqueous silver nitrate in diffuse daylight, six hours to three days, depending on thickness of smear and need for haste.

3. Correct color for smear is a dull gray brown with metallic sheen—wash in water, dry and examine. Spirochetes are deep brown or black, cells delicate brown.

Microscopic objects are measured by viewing with an ocular fitted with a graduated glass disc. Their values are indicated on the apparatus.

CHAPTER VI

BACTERIOLOGICAL LABORATORY TECHNIC

In order to study bacteria by other methods than the simple examination of their morphology by means of stains, and by the hang-drop, or block method, they must be cultivated either in the bodies of experiment animals, or in culture media artificially prepared. The latter method is the most widely used in laboratories. It is necessary, in order to study bacteria, that the media shall not contain any extraneous bacteria to begin with, and that they shall be cultivated under such conditions that such bacteria cannot reach the media at any time. To accomplish all this, the culture media must be kept in glass vessels, such as test-tubes and flasks that have been sterilized. And, since all animal and vegetable substances, not actually alive, are teeming with a multitude of bacteria, these substances must be sterilized too, in order that the media shall be free from any living organisms.

Glassware is cleaned by boiling with soap suds or powder or if very dirty by immersion in saturated watery solution of bichromate of potash plus an equal part of sulphuric acid. This latter must be very carefully washed away in running water.

Glassware, such as pipettes, Petri dishes, flasks and test-tubes, are sterilized best by dry heat in hot-air sterilizers. The apparatus is subjected to a temperature of 150°C . for one hour, or until the cotton plugs are slightly brown. The glassware should be put in wire baskets and the test-tubes should be kept erect. Petri dishes are best sterilized in a wrapping of paper. Flasks and test-tubes are always plugged with raw cotton, which prevents the ingress of bacteria, while air can reach the media through it freely.

Sterilization of culture media is accomplished in steam sterilizers of two patterns; of these, the autoclave, using steam under pressure, is the most satisfactory and is most generally used at present.

The baskets containing the culture media are placed in the autoclave after sufficient water has been put in it. The instru-

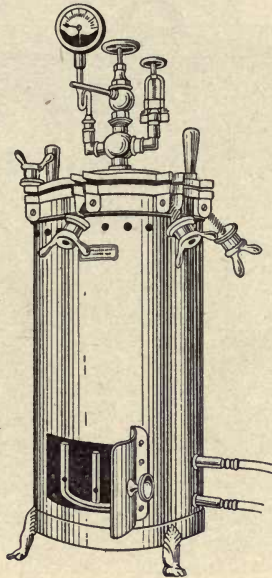


FIG. 22.—Autoclave.

ment must never be allowed to run without water. The lid is screwed down and the flame started; free flowing steam should escape from the valve before the latter is shut. When the pressure has risen to 1 atmosphere (15 pounds) or 120°C . and held there for twenty minutes, all bacteria are destroyed, and the media can be safely assumed to be sterilized. If media containing sugar or gelatine are to be sterilized, the temperature should not run above 110°C ., since, if this is done the gelatine will

not solidify when cold, the sugar is caramelized and the media blackened.

Potato tubes are harder to sterilize at times, and it is safer to repeat the operation in twenty-four hours.

Fractional method of sterilization, or Tyndallization, is accomplished by heating the media to 100°C . on three successive days

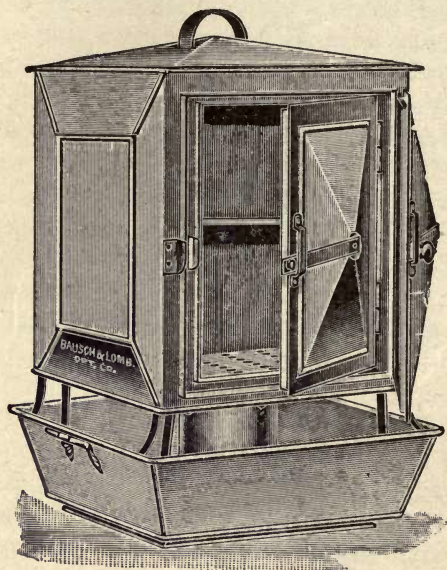


FIG. 23.—Arnold sterilizer.

in a Koch or Arnold sterilizer. By heating culture media to this temperature, all the vegetative, or adult, forms are killed, while the spores are not affected; after the first sterilization, at room temperature, the spores vegetate and become adult bacteria, when on the second sterilization they are non-resistant to 100°C . and are killed. Spores remaining after this develop into adult forms again and are killed on the third day, at the third sterilization. This fractional sterilization is employed under many

circumstances, and is certainly the best for media containing carbohydrates of any kind. To be effective, the media must be exposed to a temperature of 100°C . for thirty minutes, that is, thirty minutes after the steam has begun to form. Overheating of sugars causes them to caramelize and turn black.

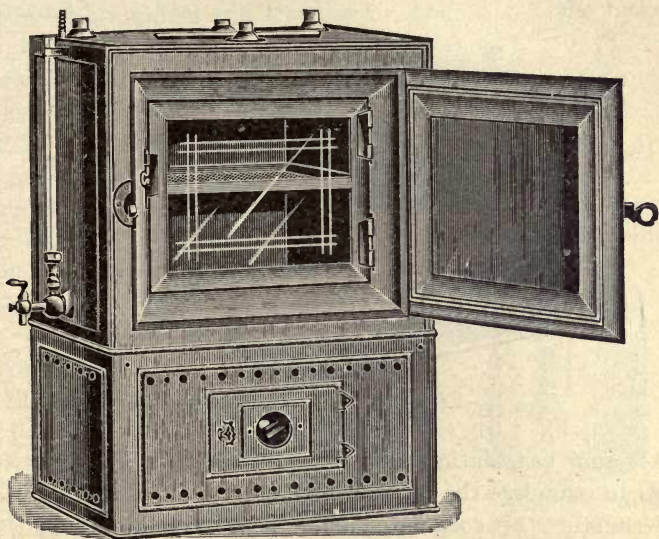


FIG. 24.—Incubator.

Bacteria that grow best at a temperature of 37°C . (most of the pathogenic ones do) develop more rapidly and luxuriantly in an incubator, or thermostat. Indeed some organisms, like the tubercle bacillus, cannot be cultivated without it. An incubator comprises an air chamber surrounded by a water chamber, and this, in turn, is surrounded by another air chamber. It is essential that the interior of the incubator be kept at an even, unvarying temperature. This is accomplished by using a small bunsen flame under the incubator. The heat from the flame warms the outer air chamber or jacket, and it in turn warms the water

jacket, and the interior air chamber, where the cultures are kept, is thus heated to the required temperature. The amount of heat is automatically regulated by a thermo-regulator, which diminishes the gas supply if the temperature runs too high, or increases it if it runs too low. The Roux regulator is the simplest and most efficient one.

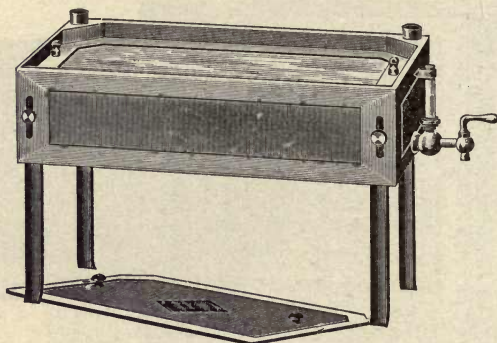


FIG. 25.—Blood serum coagulating apparatus.

A serum coagulating apparatus is needed in laboratories in order to coagulate the tubes of blood serum (Fig. 25).

Serum tubes are coagulated in it at a temperature of about 70°C . They are then sterilized by heating them either by the fractional method or in the autoclave.

The separation of bacteria from the bouillon in which they grow for the preparation of toxins requires the use of a bacteria or germ proof filter, the best type of which is the Chamberland or Pasteur unglazed porcelain filter. These filters are of varying grades of fineness, and are so made as to be easily sterilized. The common pathogenic bacteria cannot pass through the pores of the ordinary filter, but toxic agents are known to pass through the finest filters, though they cannot be discovered, as they are submicroscopic.

To operate the porcelain filter it must fit into the neck of a

vessel very tightly, so that a vacuum may be maintained in the latter by means of an air pump.

Collodion sacs are sometimes used in animal experiments. Bouillon cultures are placed within the sacs, which are then inserted in the abdomen of an animal and left there. The sac is made of collodion because it is non-absorbent and allows the bacterial juices and products to osmose outward and be absorbed by the animal, while the animal fluids percolate into the sac. There are several very ingenious ways of making these sacs, but the details are too elaborate to be described here.

BOUILLON

Bouillon or broth is the most useful of all the nutrient media, since it is not only used as a liquid medium, but by the addition of gelatine, or agar, it is converted into solid media.

There are two methods of making bouillon:

Method 1.

Take 500 grams of lean beef free from all fat, chop it fine and cover with 1,000 c.c. of water, shake and place on the ice overnight. Then squeeze the fluid out of the meat by means of a cloth, and supply enough water to make a litre. Inoculate this meat juice with a fluid culture of the colon bacillus for the purpose of fermenting the meat sugar. For this purpose the inoculated juice is allowed to stand at room temperature overnight. Bring to a boil and add

10 grams of Witte's peptone.
5 grams common salt.

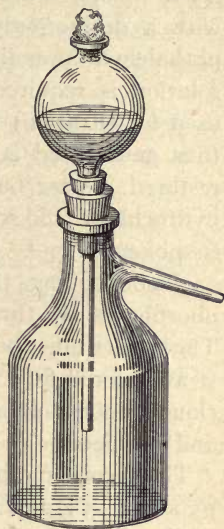


FIG. 26.—Kitasato filter for filtering toxins. (Williams.)

Weight the saucepan and contents and heat to 60°C. Supply the water lost by evaporation. Neutralize either by adding sufficient sodium hydrate, 10 percent solution, until red litmus paper is colored a faint blue, or else titrate 10 c.c. of the mixture with a decinormal solution of sodium hydrate, using phenolphthalein as an indicator, and after finding how much of a normal solution is required to neutralize 990 c.c. (1,000 c.c.—10 c.c. used for titration) this normal solution is added. The mixture thus neutralized is then boiled for five minutes and the weight restored. After boiling, from .5 percent to 1.5 percent normal hydrochloric acid solution is added and the acidity thus produced is spoken of as + .5 percent or + 1.5 percent as the case may be.

Upon boiling, the albumins are coagulated by heat, and the phosphates are thrown down. The acid re-dissolves the latter. The former must be removed by filtration. The filtrate is a clear straw-colored fluid of an acid reaction which should not become cloudy upon boiling. This is then run into flasks or test-tubes and sterilized.

The second method is much more convenient, and is prepared by adding 3 grams of Liebig's beef extract to a litre of water, and adding the peptone and salt, as in the previous method, and proceeding as before. To filter the bouillon, the filter paper must be folded many times, and the funnel must be carefully cleaned.

Newer methods for the titration of media have been constructed on a physico-chemical basis, attempt being made to estimate exact reaction in terms of ionic dissociation and hydrogen concentration. Distilled water contains ionizable hydrogen according to the following formula $1 + 10^{-7} = \log. - 7$, a mathematical statement abbreviated for convenience to 7., or the symbol of strict neutrality and called the Ph or hydrogen concentration. As the ionization of hydrogen increases, acidity becomes greater and the numerical factor drops to 6.8 or lower; as the hydrogen value decreases the figure rises. Substances in solution, such as salts or organic matter act as buffers or agents which attempt to

keep the hydrogen ion concentration fixed. Dilution does not change the concentration. Mixtures of acid and alkaline salts which will give hydrogen ion concentration ranging from Ph values of 5. (acid) to 9. (alkali) have been prepared for use in bacteriology. To these solutions may be added dye indicators. Different dyes have different ranges of color changes. The most useful are the following:

		Range of Ph
Brom cresol purple	yellow-purple	5.2 — 6.8
Brom thymol blue	yellow-blue	6.0 — 7.6
Phenol red	yellow-red	6.8 — 8.4

Litmus which has so long served a useful purpose in the laboratory has a Ph of about 6.8. A convenient method for practical use in the laboratory is as follows:

Materials:

1. Chemically clean test tubes.
2. Freshly distilled water.
3. N/15 KH_2PO_4 (primary phosphate) solution. (1.078 gm. to litre of water.)
4. N/15 Na_2HPO_4 (secondary phosphate) solution (11.996 gm. per litre of water.)
5. Phenolsulphonphthalein .01 percent water solution (or phenol red).
6. N/20 NaOH, N NaOH.
7. N/20 HCl, N HCl.

Phosphate mixture for Ph values 7.0 to 8.0.

Ph value	Amt. of primary phos. in c.c.	Amt. of secondary phos. in c.c.	
7.0	19.4	30.6	Quantities to be mixed to get indicated Ph value. 5 drops indicator to each.
7.2	14.2	35.8	
7.4	9.7	40.3	
7.6	6.5	43.5	
7.8	4.3	45.7	
8.0	2.5	47.5	

Procedure.

Add 20 c.c. freshly distilled water to chemically clean test tube. Add 10 drops phenol red and 5 c.c. of medium to be titrated. Compare with standard.

Titrate with $N/20$ NaOH or $N/20$ HCl to match tint of standard tube corresponding to the Ph desired. Calculate the amount of normal alkali or acid to be added to the medium to give the proper reaction.

If the natural color of the medium makes it difficult to match with the standard solutions, a tube of 20 percent solution of the medium in water may be placed back of the standard solution. The best generally useful reaction is 7.4, a figure suitable for most pathogenic organisms.

GELATINE

To make **gelatine**, bouillon is made to which gelatine is added in order to render it solid. The following steps are taken:

- (a) Take a litre of water in a saucepan and add chopped beef or beef extract as in bouillon. After standing overnight squeeze the beef and extract the juice.
- (b) Add 1 percent peptone, .5 percent salt, 10 percent to 15 percent best gelatine and weigh.
- (c) Heat until ingredients are all dissolved.
- (d) Neutralize, gelatine is highly acid and requires much alkali.
- (e) Boil five minutes and restore weight, boil till albumin coagulates.
- (f) Cool to 60°C . and add an egg well beaten up in water.
- (g) Boil slowly till all the egg is coagulated. This clears the medium of fine particles that are not removed by filtration. Add .5 percent normal hydrochloric acid.
- (h) Filter through absorbent cotton on a funnel previously wet with boiling water.
- (i) Tube and sterilize in autoclave for fifteen minutes at 110°C . Litmus, or lacmoid, or neutral red may be added to the gelatine as an indicator.

AGAR-AGAR

To make agar :

- (a) Take 20 grams of powdered or chopped agar.
- (b) Add to 500 c.c. of water, place in a can in autoclave and heat to 120°C. Then cool.
- (c) Add this to 500 c.c. of bouillon of double strength, making 1,000 c.c.
- (d) Neutralize.
- (e) Cool to 60°C.
- (f) Add the egg to the mixture, stir.
- (g) Boil till egg is coagulated thoroughly.
- (h) Titrate and adjust to desired acidity as given under bouillon, and while boiling hot, filter through absorbent cotton wet with boiling water.
- (i) Run into tubes. Sterilize. Slope the tubes for twelve hours and store in dark place.

To make glycerine agar add 5 percent of glycerine to the agar before neutralizing. To make agar for tubercle bacilli, veal bouillon may be employed, and glycerine must be added.

Litmus Milk

Carefully skimmed milk, to which litmus tincture has been added, is run into tubes and sterilized. This is a valuable culture medium. It is also a reagent.

Potato Tubes

1. Wash some large potatoes and with a Ravenel potato cutter, cut out semi-cylinders of potato. Immerse in running water overnight, in order to prevent them from turning black. It is well to wash these bits of potato with 1-10,000 bichloride of mercury six hours and running water over night. Some laboratories soak their slices in sodium carbonate solution. It is desirable to know

the reaction of the medium and each batch should be tested, then marked whether faintly or strongly acid or alkaline.

Thrust absorbent cotton to the bottom of the tube and wet with distilled water; place the potato upon the cotton, then plug the tube and sterilize in autoclave twice. The tubes should be sealed.



FIG. 27.—Potato in culture tube. (Williams.)

PEPTONE SOLUTION—Dunham

Take Peptone.....	10 grams.
Salt.....	5 grams.
Water.....	1,000 c.c.
Mix. Boil. Filter and store in tubes and sterilize.	

This is used to demonstrate the production of indol. Reaction should be neutral.

SUGAR MEDIA

One of the most important parts of determinative bacteriology is the discovery of the different fermentative powers upon carbohydrates of otherwise similar germs. Monosaccharides (dextrose and galactose), disaccharides (lactose, saccharose), alcohols (glycerine mannite) and some starches (dextrin, inulin) are in common use daily in the laboratory to show the enzyme action of various species, indicated by acidity and gas production.

One percent solutions of these carbohydrates are made in neutral broth or agar. The best method is to prepare a 20 percent solution of the material, sterilize it in the steam sterilizer and add from this to the stock medium sufficient to make the required percentage. This avoids repeated heating. The media should be neutral. Addition of litmus tincture or Andrade's indicator (100 c.c. of .5 percent watery acid fuchsin decolorized by the addition of 16 c.c. N/1 NOOH) will supply an index for acid production.

BLOOD AGAR

Is prepared by adding to melted agar sterile defibrinated blood of any animal in the proportion of 1 of blood to 5 of agar. The mixture may then be allowed to harden in a slanting position or poured into plates.

BLOOD SERUM

The blood of a dog, sheep or cow drawn under strictly aseptic precautions is collected in a sterile jar and after the serum has separated, it is run into tubes by sterile pipettes and simply coagulated by heat. Sterilization is not necessary, and is harmful for the growth of the tubercle bacilli, because salts are formed which interfere with the growth of the bacteria. If this serum be mixed with 3 or 4 parts of distilled water and sterilized five days in the Arnold at 70°C. it will be a slightly turbid, opalescent liquid very suitable for many organisms, particularly cocci. This medium with the addition of bits of animal tissue is a good medium for spirochætes.

LÖFFLER'S BLOOD SERUM MIXTURE

Blood serum of an ox, a sheep or a horse is employed, mixed with bouillon containing 1 percent of grape sugar.

Seventy-five percent of blood serum is mixed with 25 percent bouillon. This is run into sterilized tubes and the latter are placed in a blood serum coagulator and coagulated in a sloping position at a temperature of 65°C. or thereabouts.

After they are coagulated they are sterilized by heating an hour each day at 65°C. five successive days, or at 95°C. for an hour on three successive days. After sterilization the tubes should be sealed carefully.

Ascitic and hydrocele fluids may be used for this medium or in the liquid form combined with plain broth; they need no addition of sugar as they contain a small percentage.

SPECIAL MEDIA

Endo medium for the Typhoid series. Add 1 percent lactose to plain neutral agar and 1 percent of the following solution: 10 c.c. of 10 percent watery sodium sulphite and 1 c.c. of saturated alcoholic solution of fuchsin, heated in the Arnold twenty minutes. This should be mixed freshly each time since the medium is properly colorless but becomes a pale pink on standing, being then useless. It is poured into plates and allowed to cool and harden thoroughly before use.

Russell's Medium.—Plain neutral agar receives 1 percent lactose, .1 percent glucose and an indicator, either litmus or Andrade. It is used as slants with a deep butt.

The first medium is to distinguish typhoid colonies from colon bacillus; pale bluish round colonies versus irregular red ones. The Russell medium gives with typhoid, a colorless surface growth and acid in the butt; paratyphoids give bubbles of fermentation in the butt; colon makes distinct red slant growth and much gas.

Eggs are employed as culture media. The yolks and whites of a number of eggs are shaken together in a flask and then strained through a towel to remove the froth. The mixture is then run into tubes and coagulated and sterilized like blood serum. On this mixture the tubercle bacillus grows very well.

These are the common culture media used in laboratories. For a more technical description of the manufacture of these and other media, the student is referred to books devoted to laboratory technique.

Litmus tincture is made by adding a large handful of litmus cubes to a pint of water and boiling down to one-fourth its volume. This is then filtered through paper and stored after sterilization.

The Study of the Growth of Bacteria—Cultures

Bacteria growing in groups on culture media are spoken of as colonies. Aerobic bacteria may be made to grow on culture

media by simply inoculating the media with some pus or blood containing them, by means of a sterile pipette or platinum needle. But such cultures are made up of colonies of different sorts of bacteria—some pathogenic, some non-pathogenic, etc. To separate the various bacteria so that they will grow in isolated groups, is a comparatively easy matter, and is accomplished in several ways. The simplest is to employ several tubes of agar or blood

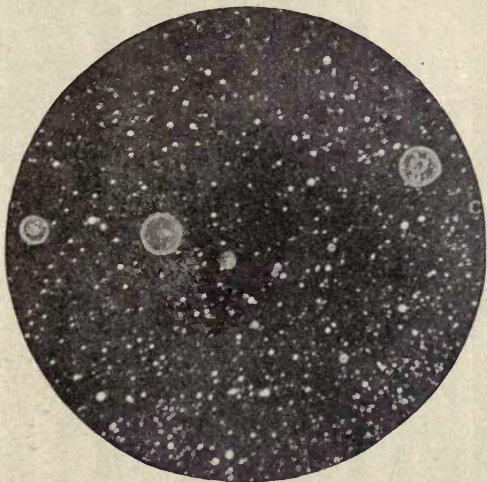


FIG. 28.—Colonies in gelatine plate showing how they may be separated and the organisms isolated. (Williams.)

serum. Over the surface of each of these, a platinum loop containing pus, or other matter, is rubbed successively. These tubes are then incubated. After a few hours, the first one exhibits a copious growth of many different kinds of bacteria growing confluent together, from which it is impossible to isolate any pure cultures. The second tube is less covered with bacteria while, the third, instead of containing a mass of bacteria, exhibits tiny little dots, or colonies (pure cultures) growing discretely isolated. By means of a sterilized platinum needle these little colonies

may be fished out and transplanted to fresh culture tubes, and after a few hours' growth they become pure cultures. An old method employed in many laboratories, in breweries and originated by Pasteur was what is known as the dilution method.

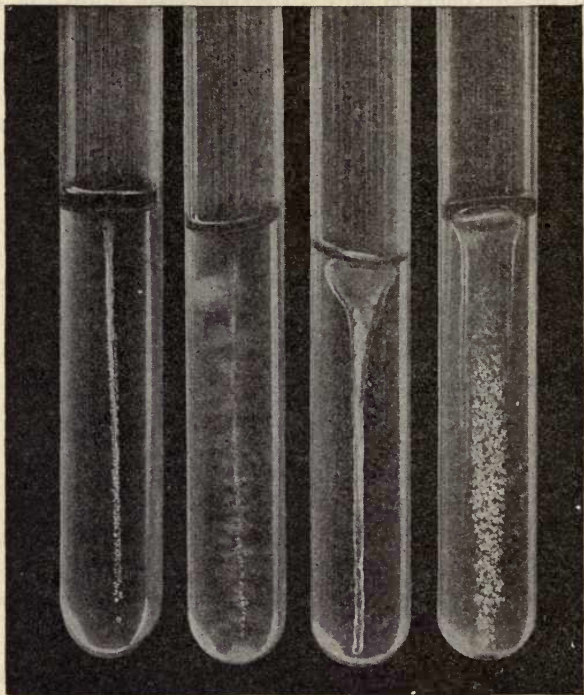


FIG. 29.—Series of stab cultures in gelatine, showing modes of growth of different species of bacteria. (Abbott.)

Numerous flasks are inoculated by matter containing bacteria very highly diluted in bouillon and by means of a sterile pipette drops of this highly attenuated mixture are dropped into flasks of sterilized bouillon or wort. Most of the flasks will show a mixed growth but a few will show only one kind of organism.

Another method is to inject some matter containing pathogenic bacteria into a rabbit or guinea pig. The various juices and the leucocytes of the animal destroy the non-pathogenic bacteria and a pure culture, of a pathogenic form, may be isolated from the blood or pathological lesions at autopsy and transferred to culture media.

By far the most useful and ingenious method of procedure is the Koch, or plate method. Koch was the first to employ solid culture media for this purpose, and his method depends upon the principle that a liquid culture media may be inoculated with bacteria and then spread out on sterile glass plates or dishes where it quickly hardens, the bacteria being uniformly separated from each other, and for a time at least kept isolated by means of the solid media, and after they have developed into isolated colonies they may be transplanted to tubes of media in which they may be stored. In another way if a man wanted to secure a pure lot of seed of a single variety from a multitude of many kinds, it would perhaps be impossible to pick out by hand the seed wanted because of their fewness and smallness, but if he sowed them and waited until the plants developed they could then be identified and gathered (Abbott). Thus it is with plate cultures.

To isolate a pure culture of bacteria, say the *Bacillus pyocyaneus* from pus, the following procedure is adopted in this method.

Three sterilized petri dishes, and three tubes of agar or gelatine melted at 40°C. are used. A loopful of pus is taken up by a sterilized platinum loop and mixed with the gelatine of the first tube. To do this the tube is held across the left hand in a horizontal

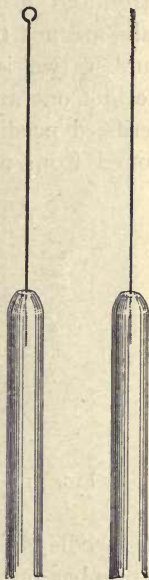


FIG. 30.—Needles used for inoculating media.

position and the cotton plug is removed, and held by its outside end between the fingers of the left hand, care being taken to prevent the tubal part of the plug touching anything and being contaminated. The platinum loop is then slowly and carefully introduced into the median, and stirred around so that the tube walls are not touched. The needle is again sterilized and tube number two is held in the palm of the left hand parallel to the first one and its plug is removed also; then with a carefully sterilized needle, three loops of the inoculated gelatine are removed from number one and mixed with number two tube.

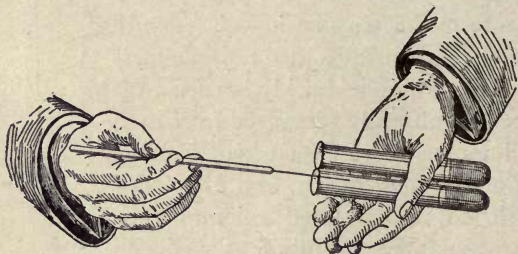


FIG. 31.—Method of inoculating culture media. (Williams.)

The needle is then again carefully sterilized in the flame, the plug of number one is carefully replaced and another tube, number three, is held in the palm of the left hand and its plug is carefully removed and held as the previous ones were. With the sterilized loop three loopfuls of the gelatine from number two are carefully introduced into number three and the needle is then sterilized and put aside. The petri dishes should now be laid on a cold level slab, and the contents of the tubes run into the different dishes. Tube number one is taken first; the lip of the tube is wiped with the cotton plug and then held in the flame to destroy all bacteria clinging to it. The lid of a petri dish is carefully and partially lifted and the contents of the tube rapidly and evenly poured over the bottom of the plate, and the lid quickly replaced.

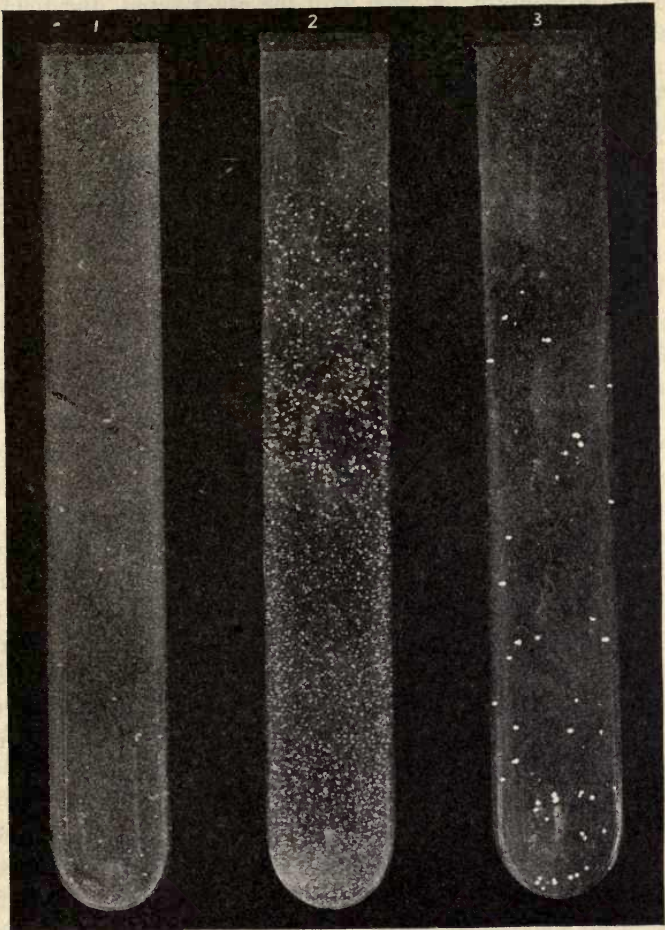


FIG. 32.—Dilution method of making cultures. 1, Is first tube containing great number of colonies; 2, contains less number; 3, relatively few. (Williams.)

This procedure is followed with the other tubes, and then the plates or dishes are put in a cool dark place, and the tubes are put into a solution of bichloride of mercury, or into boiling water.

The plates should be examined from time to time. After several days a perfect cloud of round colonies are seen in number one; a large number in No. 2 and a much fewer number, say fifty, in No. 3. It is an easy matter then to pick out a colony that is surrounded by a bluish-green halo and transfer it to a tube of agar or bouillon. In the case of pus it is more than probable that the colony is that of the pyocyaneus bacillus, and that it contains nothing but these bacilli. It must be studied in a dozen ways, before it is certain that it is this bacillus, but the preceding method is a necessary primary step to secure this organism in pure culture and may be taken as a pattern for all plate methods.

Agar plates are usually used since they have this advantage—they do not melt at 37°C . incubator temperature. When agar is used it must be melted at 100°C . and cooled below 48°C . and above 43°C . Above 48°C . bacteria may be killed. Below 43°C . the agar begins to harden, so this method must be performed quickly; the plates should be slightly warmed, the culture poured on and the agar hardened, they must be inverted in the incubator, since the water of condensation forming in the lids of the plates often falls and washes one colony into another. When gelatine plates are made, they must be kept at 20° – 25°C . It is often of advantage to cool the plates by means of ice, before they are filled.

The so-called "Stroke plates" are extremely useful for hospital bacteriology. The agar is softened, poured into plates, allowed to harden and the material to be examined is smeared upon the firm surface by a flattened platinum rod, a "Spatula." The separate colonies develop along the lines of spread and can be isolated to individual tubes as given above.

Roll Culture.—Instead of pouring out the contents of the inoculated tubes the gelatine may be made to harden on the walls of

the tubes by quickly rotating the tube in a groove melted in a block of ice. The centrifugal force distributes the gelatine over the glass, and the ice hardens it rapidly while in contact with the glass. Such tubes are veritable plates, and in them colonies of bacteria often grow as well as on the plates and may be fished out.

The various characteristics of bacterial growth may be studied in cultures. Some organismal cultures grow rapidly and luxuriantly; some discretely and slowly; colors and odors are produced by some; gelatine is liquefied by many, while others do not liquefy gelatine. Milk is curdled and digested by some; gas and acids produced by others. These various characteristics enable us to identify and differentiate bacteria.

The cultivation of bacteria in the laboratory has for its purpose a demonstration of their vital activities. This may indicate only their botanical character or it may show their relation to disease. In order that we may classify germs systematically certain criteria have been established which when added together permit us to identify and name the organisms. This is called determinative bacteriology. The principal characters to be noted are complete morphology, staining characters, particularly with Gram's method, colonial growth on agar and gelatine, potato, blood serum, milk, sometimes inorganic salt solutions, the enzymic products as indicated by fermentation of carbohydrates and solution of proteins like milk curd and gelatine. With this last comes ammonia and nitrite productions. The optimum temperature and media, and resistance to physical and chemical agencies must be taken into consideration. For pathogenic bacteria we establish as far as possible the relations with lower animals. This includes, of course, the production of soluble toxins and endotoxins.

The chemical activities of many bacteria are well displayed in litmus milk culture in which decolorization, acid or alkali formation, coagulation and clot digestion are the important ones.

The property of converting sugar into acids and gases is best studied in fermentation tubes.

Into sterile fermentation tubes bouillon containing sugar is run, these are plugged and sterilized. They may be inoculated with bacteria and if gas production occurs it is quickly manifested in the closed arm. The component gases may be studied and the various properties determined. This gas ratio is of use in identi-

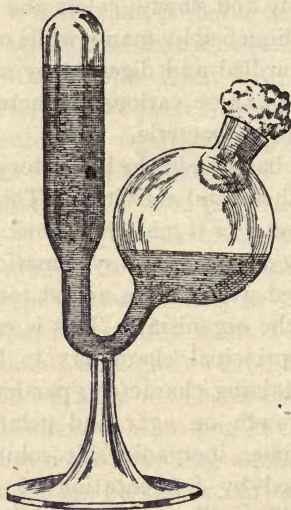


FIG. 33.—Fermentation tube. (Williams.)

fying various bacteria and differentiating them. The closed arm of the tube being shut off from free air by the amount of bouillon in the open arm is practically an anaerobic tube and is employed for this purpose. Bacteria that grow only in the closed arm are considered anaerobes. By inoculating a gelatine tube with bacteria while it is melted and then letting it solidify, previously shaking the tube vigorously, gas formation will be speedily mani-

fested by the presence of bubbles. Acids are detected in cultures by the employment of various indicators in the culture media. Litmus, lacmoid, andrade, and neutral red are used for this purpose. By titrating bouillon of previous known acidity with a decinormal soda solution, the amount of acid produced by different bacteria can be estimated.

Various sugars are fermented by bacteria, and lactic, acetic, and butyric acids are produced. Indol is also produced by many bacteria (colon bacillus, cholera bacillus), and its presence in culture is an important means of identifying different bacteria. The organism to be studied must be grown in culture media known to be free from indol. For this purpose, all meat extracts must be excluded and a simple solution of peptone and salt, run into tubes and sterilized, is used. After bacteria have grown in this media for several days the indol produced, if it is produced, is detected by adding a few drops of pure sulphuric acid. If a red color (nitroso-indol) is not produced, a few drops of sodium nitrite solution (.02 gram to 100 c.c. of water) must be added, and if a pink to deep red color does appear it may be safely assumed that indol is present.

Ammonia is detected in culture by suspending a piece of paper wet with Nessler's reagent above a bouillon culture of a given organism. If a yellow to brown color is produced ammonia is present.

Nitrites are detected by growing the organism in a solution of a nitrate (.02 gram potassium nitrate, 10 grams peptone in 1,000 c.c. of water).

Incubate for a week and then add 1 c.c. each of the following solutions:

(a) Sulphuric acid.....	.5 gram.
Acetic acid.....	150 c.c.
(b) Amido naphthaline.....	.1 gram.
Water.....	20 c.c.

Boil, filter, and add 180 c.c. of dilute acetic acid.

If nitrites are present a pink color is produced by these reagents. Enzymes may be detected by noting whether gelatine is liquefied, or milk curd digested. Both these actions are evidences of the presence of enzymes.

Bacteria growing exclusively in the absence of oxygen are known as anaerobes; to cultivate these successively various forms of apparatus are necessary.

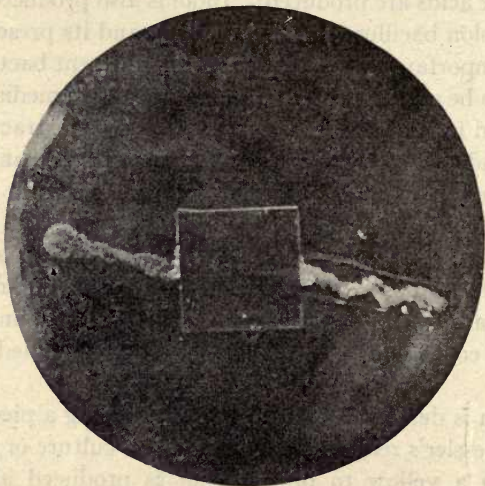


FIG. 34.—A streak made in agar by a needle inoculated with aerobic bacilli and then covered at one spot with cover-glass. The aerobic organisms will not grow in the anaerobic conditions under the glass. (Williams.)

The following methods are pursued in ordinary laboratory manipulations:

1. Exclusion of oxygen.
2. Exhaustion of oxygen by means of an air-pump.
3. Absorption of oxygen by means of chemicals that absorb oxygen from the air. A mixture of pyrogallie acid and sodium hydrate absorbs oxygen rapidly, leaving nitrogen only in the chamber.

4. Displacement of air by means of an air-pump and allowing hydrogen to enter the vacuum.

Under the *first* method we may either exclude oxygen by laying sheets of sterile mica or a cover-glass on the surface of the agar or gelatine plates (Fig. 34), thus excluding air, or deep punctures may be made in tubes half filled with gelatine or agar, for growths often occur in the depths of the medium, especially if

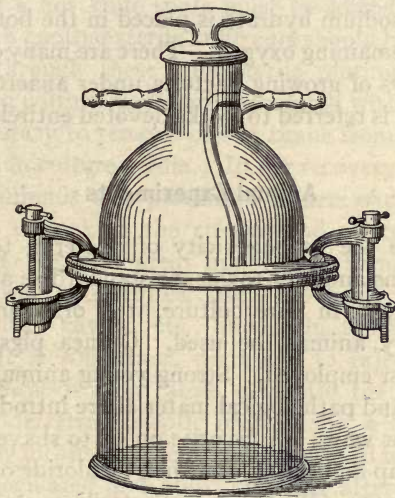


FIG. 35.—Novy jar.

the latter has been boiled previously to expel the oxygen; or, instead of mica, sterile paraffine may be poured over the top of the tube. The layer of paraffine excludes the air. Flasks filled with bouillon, or tubes filled with bouillon, or melted agar may be inoculated with an anaerobic culture, but the filling of the vessel with the medium must be absolute so that no space is left for air, otherwise the organisms may not grow. Roux employs a long sterile glass tube, which he completely fills with melted agar

inoculated with the organism he wishes to grow. The ends of the tube are then sealed in a bunsen flame and there being no air, anaerobic conditions are fulfilled, and organisms grow. After colonies appear the tube is broken at a file-mark near the colony and tubes inoculated therefrom.

Under other methods large Novy jars are used for the reception of petri dishes and test-tubes. From these jars the air is withdrawn, and hydrogen allowed to flow into it. A solution of pyrogallic and sodium hydrate is placed in the bottom of the jar to absorb any remaining oxygen. There are many other ingenious mechanical ways of growing bacteria under anaerobic conditions and the student is referred to works devoted entirely to technique.

Animal Experiments

To determine the pathogenicity of bacteria; to measure the strength of toxins and anti-toxins, to standardize anti-toxins, and to recover bacteria in pure culture, it is often imperative that small laboratory animals be used. Guinea pigs, rabbits, and mice are oftenest employed. Strong young animals are the best. Culture toxins and pathological material are introduced into their bodies in various ways. A favorite one is to shave the abdomen, scour it with soap and water, and then bichloride of mercury, and finally sterile water. With a pair of sterile scissors a small hole is cut in the abdominal parieties and through it a loop containing a drop of culture is run into the peritoneal cavity, or under the skin. With ordinary fluid material, a syringe may be used to inject it directly through the abdominal wall. The animal is carefully weighed, and it is watched from day to day. If it dies an autopsy is made on it.

Other methods consist in injecting fluid culture into the veins of the ear, or into the peritoneum, by means of sterile hypodermic syringe. The autopsy should be made carefully, the animal should be thoroughly wet with a solution of bichloride of mercury,

then it should be stretched over a pan, especially devised for the purpose, or nailed to a board. The skin over the abdomen and thorax must then be shaved and sterilized with a solution of bichloride of mercury. The walls should then be seared in a line from the throat to the pubes with a hot knife, and through this line a cut should be made opening up the thoracic and abdominal cavities.

By means of a hot knife spots must be seared on the various organs, and with another sterile knife cuts should be made into the organs, then through these cuts sterile platinum needles are thrust, and then culture media are inoculated with them. Sometimes it is necessary to remove bits of tissue from various organs and place them in culture media. In the recovery of the tubercle bacillus from animals this procedure is necessary. Great care must be taken in making the culture and all tubes should be carefully stored. It is of great importance to make smears on cover-slips as well as cultures, from the heart cavities, liver, kidneys, peritoneal cavity, etc., and stain them directly with Gram stain. It is sometimes necessary to inject cultures, or bits of nerve tissue from a rabies case into the brain. To do this, remove under strict aseptic precautions, a button of bone from the skull by means of a trephine. It should not be forgotten that animals inoculated and killed or dying after infection may present dangerous material to the laboratory personnel. After an autopsy, a strong disinfectant should be generously spread over all parts of the animal and autopsy tray.

Histological Methods

Sections of tissues from infected animals are often examined and stained by appropriate methods. To demonstrate bacteria, the tissues should be hardened in alcohol or formaldehyde solution (4 percent), and imbedded in celloidin, then cut into sections and mounted in the following different ways:

I. Löffler's Method.

- (a) Float section in water.
- (b) Remove with section lifter to Löffler's methylene blue from five to thirty minutes.
- (c) Decolorize in 1 percent solution of acetic acid for ten seconds.
- (d) Dehydrate in absolute alcohol for a few minutes.
- (e) Clear in xylol.
- (f) Mount in balsam.

II. Weigert's Method.

- (a) Place in lithium carmine five minutes.
- (b) Then in acid alcohol fifteen seconds.
- (c) Wash in water.
- (d) Transfer to slide and dry with blotting paper.
- (e) Apply Ehrlich's gentian violet for three minutes.
- (f) Blot and place in Gram's solution for two minutes.
- (g) Wash and dehydrate in aniline oil.
- (h) Wash with xylol.
- (i) Dry, mount in balsam and examine.

In Löffler's method all the tissues, especially the nuclei and the bacteria, appear blue.

In Weigert's method, if the bacteria stain by Gram's method, the tissues appear pink, the bacteria a deep blue-black. Tubercle bacilli are to be stained in tissues, best fixed in formaldehyde solution, by heating with carbol-fuchsin as given on page 106; the section is decolorized by 3-5 percent hydrochloric acid and cleared by passing it through 95 percent alcohol, absolute alcohol and finally xylol. It is then mounted in balsam.

Paraffine embedding methods may be employed, but for these and other methods of staining the student is referred to works solely devoted to technique. The staining methods are the same for paraffine and in experienced hands give better results.

CHAPTER VII

ANTISEPTICS AND DISINFECTANTS

Many chemical substances have the power of entering into chemical union with the protoplasm of bacterial cells and so forming new compounds, and coagulating the protoplasm; other chemicals dissolve the bacterial bodies.

Bacteria differ in their powers to resist these agencies; the anthrax spore is much more difficult to kill than the typhoid bacillus; these chemical substances act better at a high than a low temperature.

A chemical disinfectant, such as copper sulphate, acts more rapidly and effectively in a watery solution than in a complex albuminous one.

Park's division of the change of viruses under the influence of chemicals is convenient and instructive. Attenuation is the temporary restriction of growth, but especially of virulence and pathogenicity; these are resumed upon cessation of action of the chemical. Antisepsis is a definite restriction of growth but there is no destruction. Incomplete sterilization is the destruction of vegetative forms of bacteria but not spores. Disinfection or sterilization is the destruction of all disease producing virus.

It is often necessary to determine the exact minimum amount of an antiseptic that will destroy a given organism or produce a complete inhibition of growth; for this purpose small amounts of a disinfectant are added to gelatine in test-tubes and these are poured into plates and the result noted.

Previous to pouring the plates each tube is inoculated with a loopful of culture and thoroughly mixed with the medium.

Another method is to make bouillon cultures of an organism and add to each a certain percentage of the solution of the antiseptic, and abstract every few minutes after the addition of the chemical one loopful of the mixture and inoculate fresh media.

Pieces of thread sterilized, and then put in fluid cultures may be used in experiments; they are dipped into solutions of chemicals for varying lengths of time and then placed in culture media and growth noted.

It will be found in the case of most antiseptics in dilute solution that an interval of time must elapse before the organisms are killed. This is determined by observing the cultures made from the mixture. After five minutes, growth may occur, but after one hour, all may be dead, or it may take two or three hours.

The student should refer to works on hygiene for standard methods of controlling disinfectants, for example the Hygienic Laboratory and the Rideal-Walker methods.

The most valuable chemical disinfectants are those that kill in highly dilute solution in a short time.

Bichloride of mercury is a highly efficient germicide in watery solutions; if, however, albuminous matter is present its action is inhibited very much.

CHEMICAL DISINFECTANTS

Mercury Salts.—Bichloride of mercury in highly dilute solution is a very valuable antiseptic. It dissolves in 16 parts of tepid water. It requires an acid reaction for most favorable action and the tablets now on the market are made up with some acid having no effect upon the mercury salt. In 1-100 water solution this salt will kill anthrax spores in twenty minutes. In blood, the anthrax bacillus is killed by a 1-2,000 solution in a few minutes. In bouillon the same organism is killed in a dilution of 1-40,000; in water, 1-500,000; all in the same interval of time. The presence of the albumins in the blood or bouillon, no doubt acts as a

protecting envelope about the bodies of the bacteria, being therefore unreliable for disinfecting sputum and pus. It is also more useful and powerful when it is acidulated with a 5. percent of HCl, or when it is mixed with common salt or ammonium chloride. In culture 1-1,000,000 solution prevents the growth of most pathogenic bacteria. Biniodide of mercury is said by some observers to be more powerful than the bichloride. It is certainly less likely to be interfered with by albumins.

Sulphate of copper in water is a powerful germicide. It is more potent in watery solution than in bouillon. It has a remarkable affinity for algæ and for moulds. The author found that if moulds are put into alkaline solution of copper sulphate and heated, the copper enters into chemical union with the protoplasm of the mycelia, hyphæ, and the spores; 1-400,000 of copper sulphate in water destroys the typhoid bacilli. Even nascent copper kills the typhoid bacilli, so that copper foil in drinking water has the power, after a few hours' contact, of destroying bacteria in the water.

The silver salts are useful in medicine as disinfectants, especially on mucous surfaces. The nitrate of silver is one of the most valuable of all preparations; it is about a fourth as efficient as bichloride of mercury and is not nearly so toxic. Some of the albuminates of silver are useful because of their non-irritating action.

Acids, especially the mineral ones, are valuable disinfectants in not too dilute solutions. They act chiefly as inhibitors of growth rather than destroyers of bacterial cells. In the healthy stomach, hydrochloric acid acts as a normal disinfectant, and in disease, where it is absent, it must be added in order to prevent decomposition of food. Boric acid is useful in medicine on mucous membranes.

The halogens, iodine, bromine and chlorine, are active agents for the destruction of bacteria. The cheapest of these is chlorine. It acts best in contact with moisture, since it decomposes the

molecule of water combining with the hydrogen to form free HCl and setting free oxygen.

Dry chlorine gas (45 percent) failed to kill dry anthrax spores in one hour, but when moisture was introduced 4 percent chlorine killed the spores.

"*Chloride of lime*," chlorinated lime, in 1 percent solution kills most bacteria in one to five minutes. Iodine preparations like chlorine ones are very powerful. They are of great use in medicine; ordinary tincture of iodine painted over infected areas acts as a powerful germicidal agent. It is too expensive to use in house disinfection and it is exceedingly destructive to all metallic objects. A 5 percent solution in 50 percent alcohol acts as a splendid disinfectant for intrauterine injection in puerperal sepsis. It is now said that 10 percent iodine tincture in 70 percent alcohol is the most efficacious, practical, medical disinfectant. Many claim it to have the highest penetrating powers.

Dakin's solution is a mixture of chlorinated lime and sodium carbonate in water and sodium bicarbonate, with an alkalinity of .45 percent. This mixture when in the presence of organic matter decomposes with the formation of hypochlorous acid which may further change into chlorimido—(NCl) to which changes the antiseptic action is due. The solution is used for infected wounds, being introduced by methods of infiltration and drainage. It has an irritant effect upon the skin which tissue must be protected. The germ-killing power of this solution is very high. Its use was followed in the great European war, by a marked reduction in spreading and fatal infections from lacerated wounds.

Chloramin is another product of chlorinated lime, depending on chlorin for its action. Being less toxic it may be applied to tissues or even mucous membranes in 2 percent solution but it is irritating and surrounding surfaces must be protected.

Dichloramin T. still a chlorine disinfectant, is less irritating than the foregoing but being unstable, must be suspended in an oil like

eucalyptœ, saturated with chlorine gas. It can be sprayed on a surface or pressed into a wound.

Carbolic acid is valuable as a disinfectant because of its stability. A 1-1,000 solution inhibits bacterial growth; a 5 percent solution is a thoroughly reliable disinfectant for morbid discharges; this strength is not injurious to metals or fabrics. A thorough solution should be made, and to be very efficient, 5 percent HCl should be added to it.

Cresol, *lysol* and *creolin* are useful as disinfectants, but are sometimes unreliable since perfect solution cannot always be made. The mixture of one of these substances with water is more of an emulsion than solution. Anthrax spores have been known to live for hours in creolin solutions. The value of these cresols is that when applied to a surface the water may evaporate but the germicide sticks and continues its effects. Glycerine is sometimes added to lighter phenol solutions to assist this action.

Peroxide of Hydrogen has a great reputation in medicine as an antiseptic. It kills bacteria, especially the pus cocci, in a few minutes in a 15 percent solution. A 40 percent solution will kill anthrax spores in a few hours. It is a powerful agent when fresh, and is not poisonous. It combines with organic matter and becomes inert. It degenerates if exposed to atmosphere and if it comes in contact with the ferments of the blood (hæmase).

Formaldehyde gas, CH_2O , is, by all means, the most useful, as well as the most powerful disinfecting agent that we have. In solution 40 percent in water, it is known as formaline. It has a marked affinity for organic substances and forms chemical combinations with many organic bodies. When it unites with ammonia it becomes inert until some acid frees it. It unites with iron, but other metals are unaffected. Its use in medicine is wide and varied. It is a deodorizer; renders gelatine glass-like and insoluble in boiling water. It may be liberated as a gas in apartments and ships, actively destroying all bacteria. One percent of the vapor in the air of a closed room, if the air is moist, destroys

bacteria after twelve hours. It is best to keep the room closed for twenty-four hours. It may be thrown into the room in many ways; by generators which decompose the vapor of wood alcohol, when they reach hot platinum sponges, salt, or hot copper; by vaporizing a solution by means of heat; by adding permanganate of potash to a solution of formaline; by spraying a concentrated solution over bedding, floors, and walls, then closing the apartment. It is very much more active in warm air than in cold, and when the air is moist. It has been known to destroy anthrax spores wrapped up in paper and placed under blankets. All of the pathogenic bacteria are killed by it, the *Staphylococcus aureus* and anthrax spores being more resistant than anything else. It will not kill moulds unless highly concentrated. As dilute watery and alcoholic solutions decompose they should only be used when freshly made.

Sulphur Dioxide Gas.—An old and rather unreliable form of disinfectant. It does not kill anthrax spores very readily, as it requires an exposure of twenty-four hours to a 40 percent vapor in a room. It is generated by burning sulphur in a room tightly closed, and it is much more efficient if water is vaporized in the room. It is not very penetrating, is poisonous to breathe, speedily bleaches fabrics, and attacks metal objects. It is much superior to formaline as an agent for the destruction of insects, especially mosquitoes, also to kill rats infected with plague bacilli.

Lime.—Ordinary quick lime, or whitewash, is highly germicidal. It is especially efficacious in disinfecting feces from typhoid cases. Typhoid bacilli are killed after one hour's exposure to a 20 percent mixture.

Potassium permanganate in 3 percent solution is said by Koch to kill anthrax spores in twenty-four hours. It is not so efficient a germicidal agent as supposed.

Turpentine and essential oils are efficient germicides in concentration. Common mustard rubbed in the hands is said to make them sterile.

Alcohol.—Ninety-five percent and absolute alcohols are not antiseptic for the anthrax spores, since they will live for many hours in contact with absolute alcohol. In general it is unreliable. Seventy percent alcohol is the most efficient strength.

Zinc chloride in concentration is a powerful germicide. A 2 percent solution will kill the ordinary pyogenic bacteria in two hours.

Sputum, urine and dejecta are best disinfected by heat. Chemicals often are inert because they cannot penetrate the albuminous masses of the sputum or feces. Long contact with carbolic acid acidulated with HCl is very efficient. Concentrated formaline and solutions of chloride of lime may be used, also a heavy mush of lime in water.

Boiling or heating instruments and dressings by high moist heat, as in an autoclave, is the most reliable method of rendering them sterile. The exposure of dressings to 150°C. for one hour, or boiling instruments, thoroughly cleaned mechanically, for twenty to thirty minutes makes them certainly sterile.

Disinfection of the skin is a difficult undertaking from a bacteriological standpoint. In the deep layers of the skin, and in the sweat glands and hair follicles, bacteria often exist, even after the most thorough and prolonged disinfection. The application of soap and water with a stiff brush is by all means the most valuable part of the process, since with the removal of the dirt most of the bacteria are removed. Thorough scrubbing with soap and sterile water, followed by scrubbing with a 1-1,000 bichloride solution, cleansing the nails with a sterile brush, and prolonged immersion in bichloride or permanganate of potash solution, complete the process. Modern methods, even after all this preparation, require the use of rubber gloves that have been sterilized by boiling. The faultiest part of the preparation for an aseptic operation from a bacteriological standpoint, has always been considered to be the sterilization of the hands, and if these can be covered by rubber gloves that are sterile, the fault can be surely eliminated.

Antiseptic Values (after Park).

The figures refer to the relative antiseptic powers of various agents for fluids containing organic matter.

Alum.....	1 to	222
Aluminium acetate.....	1 to	6,000
Ammonium chloride.....	1 to	9
Boric acid.....	1 to	143
Calcium chloride.....	1 to	25
Calcium hypochlorite.....	1 to	1,000
Carbolic acid.....	1 to	333
Chloral hydrate.....	1 to	107
Copper sulphate.....	1 to	2,000
Ferrous sulphate.....	1 to	200
Formaldehyde, 40 percent.....	1 to	10,000
Hydrogen peroxide.....	1 to	20,000
Mercuric iodide.....	1 to	25,000
Mercuric chloride.....	1 to	40,000
Quinine sulphate.....	1 to	800
Silver nitrate.....	1 to	12,500
Zinc chloride.....	1 to	500
Zinc sulphate.....	1 to	20

CHAPTER VII

BACTERIA

STREPTOCOCCUS PYOGENES

Streptococcus Pyogenes.

Streptococcus Erysipelatis. Chain Coccus (Fig. 36).

Morphology and Stains.—Cocci grow in catenate form of from 4 to 40 individuals to a chain. The cocci divide in a single plane,

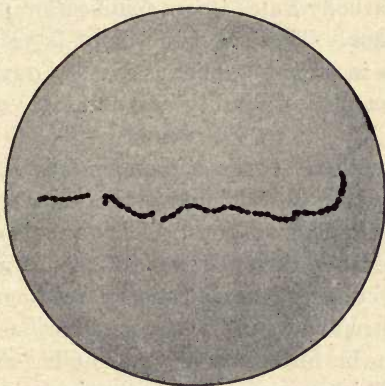


FIG. 36.—*Streptococcus pyogenes*. (Kolle and Wassermann.)

by transverse fission thus giving rise to chains. The cocci are not motile, and do not have spores. They can be stained with all basic stains, and retain the Gram's stain.

Relation to Oxygen.—They grow either in the presence or absence of oxygen, and are, therefore, facultative aerobes.

Temperature and Food Requirements.

Develop best at 37°C. Will not grow at 47°C. Never vegetate luxuriantly on any culture media, but are most prolific on

one that is faintly acid and contains animal juices like serum. They must be transplanted frequently. On **gelatine** they grow scantily without liquefaction, the growth consists of discrete little masses, while on **agar** they appear translucent colonies of very small grayish granula. In **bouillon** cultures some varieties either cloud the medium uniformly, or else sedimentate in the form of little balls, the supernatant fluid remaining clear. It ferments some simple sugars but does not form gas. In **milk** the growth is more luxuriant, and becoming acid, may be coagulated in twenty-four hours. On **potato** the growth is invisible and scanty, or absent. On **blood agar plates** colonies appear as tiny gray points with a zone of hemolysis about them. **Sugar media** of the simpler carbohydrates, show acidification.

Vital Resistance.—Thermal death-point is 54°C . in five minutes. Virulence in dried albuminous matter (pus) is retained for months. If kept on ice, vitality and virulence are retained for months also.

Chemical Activities.—*Lactic acid* and *sulphuretted hydrogen* are produced, also *ferments* which have the property of dissolving fibrin under anaerobic conditions. They are also capable of dissolving red blood corpuscles, either in culture media or in the body and about cultures on blood agar plates there is a clear halo of hemolysis, streptocolysin. They produce a strong soluble toxin, which can be filtered from the bouillon and precipitated with alcohol. This causes necrosis, anæmia and death.

Habitat.—In sewage, dwellings, dust, on the healthy human body, and in the cavities of the respiratory tract, vagina, rectum, and in the fæces. It is the cause of many diseases, *i.e.*, erysipelas, puerperal fever, meningitis, pneumonia, endocarditis, peritonitis, tonsillitis, osteomyelitis, and the diarrhœa of children.

In general septicæmia streptococcus is found in the blood, and plays an important rôle in secondary infection, causing an aggravation of the original infection, and often death. It is especially active in phthisis, scarlatina, small-pox, and diphtheria, in which

diseases it is often the cause of death. Many of the symptoms of phthisis are due to the toxins of the streptococcus; cavity formation and hectic fever for example. Its virulence can be intensified by passing it through a series of animals, until, finally, $\frac{1}{1,000}$ cu. mm. kills in one day all the mice injected with this dose. The toxin contains a peculiar hæmolytic substance, which, as before remarked, dissolves red cells of the blood, hence the anæmia in septicæmia and in suppuration. The toxin of the streptococcus, if injected under the skin, causes redness like erysipelas. Coley's fluid containing this toxin is used to treat sarcomata, since infection with the streptococcus has been known to cause a disappearance of these tumors. Practically all animals are susceptible to the streptococcus.

Agglutinations.—The serum from an animal injected with streptococci, or immunized against it, will agglutinate streptococci.

Anti-toxic sera have been prepared by injecting horses with highly virulent living culture of streptococci. The serum protects to a limited degree, and has some curative properties. Cultures of cocci from human sources have been found to produce the best toxins; there are, however, many strains.

The foregoing description represents the principal characters of the most important member of a large group of closely related streptococci. Distinctions in the group are based upon the solution of red blood cells, the fermentation of lactose, of maltose, of salicin and the coagulation of milk. The relative value of each of the members is not yet settled but investigations have enabled laboratory workers to elaborate certain techniques which may be expected to clear up the matter. The varieties now recognized are *Str. hemolyticus*, *epidemicus*, *anginosus*, *fecalis*, *salivarius*, *equinus*, and *mitis*. Some of these organisms produce no hemolysis; they come under the term non-hemolyticus and are of importance in certain respiratory and allied diseases. There is a group of organisms, a sort of connecting link with pneumococci, which produce green colonies on blood agar and

are called *Str. viridans*. These are to be distinguished from true pneumococci by the formation of a tiny rim of blood clearing in blood plates and by their insolubility in bile. These and the group of non-hemolytic streptococci are held responsible for some cases of arthritis, endocarditis, sinusitis, nephritis, etc

PNEUMOCOCCUS

Streptococcus pneumoniae commonly known as the pneumococcus, or *Diplococcus lanceolatus* (Fig. 39). (For types see page 76.)

Morphology and Stains.—This organism is usually found in the tissues and sputum, in the form of lance-shaped cocci, surrounded by a capsule. Is almost always associated in pairs, though sometimes in chains of five or six members. In albuminous fluids, or blood serum, and in milk, the organism exhibits a well-defined capsule; in bouillon and other media, it loses the capsule and the lanceolate shape, and often appears spherical, in pairs, or chains. It is not motile, has no flagella or spores, is easily stained by all the basic aniline dyes, and keeps the color by Gram's method. Under certain conditions it strongly resembles the streptococcus pyogenes, and may be differentiated therefrom by growing it on agar smeared with blood. The streptococcus causes a hæmolysis of the corpuscles, while the pneumococcus does not and the colonies are greenish.

Oxygen Relations.—It is a facultative aerobe.

Grows rapidly, but never luxuriantly at $37.5^{\circ}\text{C}.$; at $22^{\circ}\text{C}.$ much more slowly, often not at all. Grows better in the presence of serum or hæmoglobin.

Vital Resistance.—Easily killed at a temperature of $52^{\circ}\text{C}.$, exposed for ten minutes. Direct sunlight also kills it in twelve hours. While it quickly dies on ordinary culture media, it may live in dried sputum or pus exposed to diffuse light and desiccation, for four months.

Cultures.—On gelatine plate it produces very minute colonies after quite a length of time. On glycerine agar it grows better, but the colonies are small and difficult to see. In both, the colonies are whitish, with a pearly lustre. On blood serum it grows in transparent colonies. On blood agar the colonies are tiny and

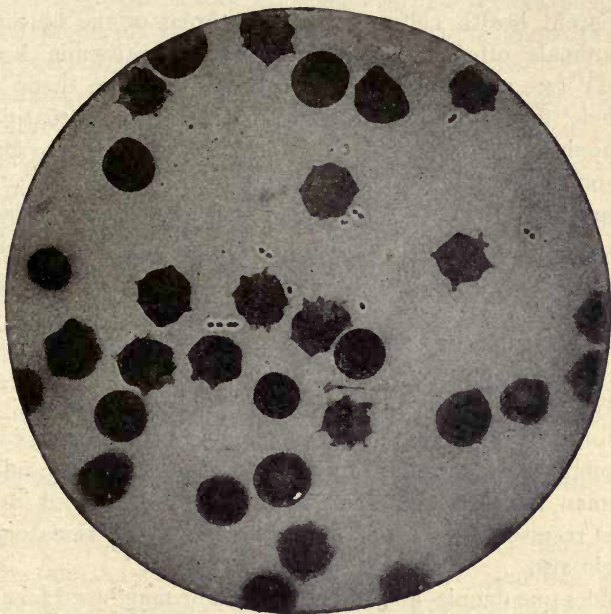


FIG. 37.—*Diplococcus pneumoniae*, from the heart's blood of a rabbit. $\times$ 1,000. (Fränkel and Pfeiffer.)

of a greenish color, lying on a brown base due to production of methemoglobin. In bouillon it grows feebly, with a whitish sediment, and in the form of chains. Here the growth is inhibited by the products of its own metabolism, *i.e.*, lactic acid. If this is neutralized by putting chalk into the bouillon the growth becomes luxuriant and the bouillon becomes thick. On potato it

will not grow. It ferments some of the sugars, the most important and characteristic being inulin. No gas is formed. Pneumococcus are soluble in bile or a solution of its salts, a distinguishing determinative character.

Habitat.—Outside the human body it has not been found, but is normally present in the mouth of about 30 percent of all people. In apparent health cultures from the throat or the sputum in animals often causes pneumococcic septicæmia because Type IV (see page 76) may be frequently found in them. The so-called "fixed types" I, II, and III occur in the throat during pneumonia but disappear shortly after recovery. It also may be found on the conjunctiva and nose in health.

Chemical Activities.—No soluble toxin has been discovered. The toxic properties are due to an endo-toxin. This organism is a pyogenic one, and causes dense fibrinous exudates on serous membranes. All tissues of the body may be attacked. Some strains of pneumococci are more neurotoxic than others.

In rabbits an intravenous injection of pneumococci will cause a septicæmia with at times areas of lobular pneumonia. The only successful reproduction of pneumonia in the lower animals is accomplished by tracheal insufflation of pure virulent cultures. In human infection the organisms are forcibly inhaled into the deepest recesses of the lungs. Pneumonia may be hæmatogenous in origin also.

Besides pneumonia, any serous membrane may be attacked and pleuritis, peritonitis, pericarditis, or meningitis may be caused. Abscesses anywhere may be due to the pneumococcus. Mucous membranes of the throat often are affected; middle ear abscesses also may be caused by this organism. Pneumococcic septicæmias are common.

During pneumonia, pneumococci may be recovered from the blood before the crisis by means of blood cultures; 10 c.c. of blood abstracted from veins is mixed with 500 c.c. of milk or bouillon and incubated. In twenty-four hours pneumococci, if present,

grow luxuriantly. Just before the crisis the organisms will not grow.

Immunity and Susceptibility.—The susceptibility of man varies greatly. Exposure to cold and hardships of various kinds predispose to pneumonia. One attack does not prevent another. It has been observed that normal leucocytes only become phagocytic toward the pneumococcus when lying in anti-pneumococci serum. It has even been noticed that these organisms grow better in the anti-serum, rather than in the normal serum. Animals have been immunized by injecting cultures and toxin. The immune serum thus produced protects small animals against infection, and stimulates phagocytosis. It has been used therapeutically in man for the cure of pneumonia with hopeful results. Oleate of soda aids in bacteriolysis of pneumococci by sera, if added to the various varieties of immune sera (see page 76). Most mammals, but few if any birds are susceptible to the pneumococci; mice, being very easily infected, are used for isolation purposes.

Agglutination of pneumococci is caused by the blood of infected individuals, even diluted at 1-60. Immune serum also has the same action.

Opsonins increase during the course of pneumonia and are at their height at or just after crisis.

Pneumococcus mucosus, also called Type III is distinguished by its large size, long chains, capsule, more generous growth in large moist colonies of not such a distinct green color and its ability to produce a serious form of pneumonia.

COCCUS OF MENINGITIS

Micrococcus Meningitidis.

Diplococcus intracellularis meningitidis.

Meningococcus (Fig. 38).

This organism is the cause of epidemic cerebro-spinal meningitis.

Morphology and Stains.—Resembles the gonococcus closely,

because it grows in biscuit-shaped pairs; is nearly always within pus cells, and like the gonococcus it is decolorized by Gram's stain. It has no spores or flagella; is not motile; grows in short chains at times, and on ordinary media best at 37°C.



FIG. 38.—Meningococcus in spinal fluid. (From Hiss and Zinsser's Bacteriology, Copyright by D. Appleton & Co.)

Relation to Oxygen.—It can be cultivated from the meninges at first best under conditions of low oxygen tension, as in a Novy jar or in an atmosphere of carbon dioxide and moisture; once growing in the laboratory it is more luxuriant in aerobic tubes.

Vital Resistance.—It is killed after ten-minutes' exposure to

65°C. and is easily destroyed by drying, and by light. It dies out rapidly on artificial culture media.

Cultures.—Best isolated on neutral semi-solid ascitic fluid or serum agar in a moist chamber. Growth is pale gray white translucent moist separate colonies. On **glycerine agar** it grows, sparingly as white viscid colonies; occasionally it develops on **potato**; thrives on **blood serum**, especially if smeared with blood, and does not liquefy the serum.

Habitat.—It is found in the pus from the meninges, sputum, and nasal mucus of persons afflicted with epidemic meningitis, or spotted fever. It has been found in the mucous membranes of healthy individuals, and these persons may be “**carriers**” of infection. After spinal puncture, it may be seen in the pus cells, and the diagnosis of the disease can be made in this way.

Virulence.—It is scarcely virulent for lower animals. If given by hypodermics into the pleura, or peritoneum, it produces death in mice. Meningitis may be, in monkeys, produced by subdural injection.

Chemical Activities.—Produces an **endo-toxin** but no soluble toxin. It is not chromogenic.

Agglutination is caused by immune serum and it is upon their property that anti-sera are standardized. It has been discovered, by means of this anti-body and by the failure of certain sera to do good, that there are several types of meningococci of different antigenic qualities. Anti-sera are made now with all available varieties.

Method of Infection.—The infection atrium of the coccus is not certainly known but most of the evidence points to the nasal passages and cribriform plate to the subdural space.

Specific Therapy is practicable; it has been discussed just above and on page 76.

There is another important Gram-negative diplococcus in the nose called **Micrococcus catarrhalis**. It is differentiated from the meningitis organism by its free growth on agar, its sugar reac-

tions and absence of active pathogenic properties. It will appear on nearly all plates made from sputum and throat cultures, in crowded city life of temperate zones. It seems not to be able to incite an infection but to continue or aggravate one already under way.

STAPHYLOCOCCUS PYOGENES AUREUS

Staphylococcus Pyogenes Aureus (Fig. 39).

Micrococcus Pyogenes.

Staphylococcus pyogenes aureus, *albus*, and *citreus* are known commonly as staphylococcus, or grape coccus. They differ only in color production on artificial media.

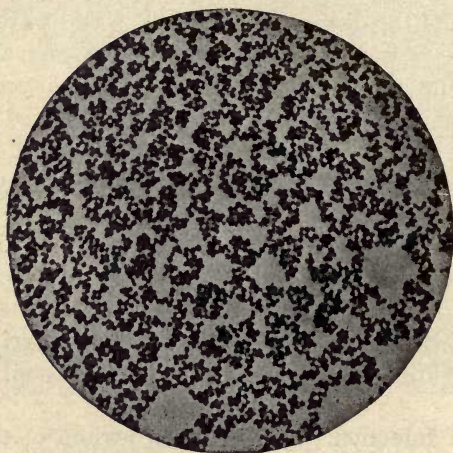


FIG. 39.—*Staphylococcus aureus*. (Williams.)

The *Micrococcus pyogenes aureus* only is here described; the other varieties have similar but much feebler pathogenic powers.

Morphology and Stains.—Round cocci, often growing in bunches like grapes. Individual cocci dividing in two planes. They stain very well with all basic dyes, and are not decolorized

by Gram's method. They are not motile; have neither flagella nor spores.

Oxygen Requirements.—The coccus grows well in oxygen, and poorly without it.

Temperature and Vital Resistance.—Thrives best at body temperature, but grows well at room temperature. Resists drying for over one hundred days in pus. Dry thermal death-point is $80^{\circ}\text{C}.$ for one hour. Moist heat $70^{\circ}\text{C}.$, kills in ten to twenty minutes. Resists freezing temperature for many months.

Exceedingly resistant to formaldehyde, more so than some spore-bearing organisms. Resists light also. It is killed by corrosive sublimate 1-1000 in fifteen minutes; 1 per cent H_2O_2 in thirty minutes.

Chemical Activities.—Produces a golden-yellow pigment only under oxygen. Generates acids, but no free gases. Creates indol and sulphuretted hydrogen; ferments urea, and produces ferments that dissolve gelatine, and the coagulated proteids of milk. The toxin is soluble in water, and acts intensely, causing violent local reaction. If in the abdominal cavity, it causes peritonitis. Subcutaneously it may produce sterile abscess, or local necrosis. There is produced in cultures a toxin having a destructive action upon leucocytes and red blood cells.

Cultures.—In gelatine it rapidly forms golden-yellow colonies, that quickly liquefy the gelatine (Fig. 40). Sterile products of the growth also liquefy gelatine. On gelatine plate, yellowish to orange colonies are formed. On agar streak a luxuriant orange



FIG. 40.—Gelatine culture staphylococcus aureus one week old. (Williams.)

growth develops. In **bouillon** there is a marked even cloudiness, with a fine pellicle on surface; moderate sediment, which upon shaking is broken up. **Milk** is rendered acid and curdles very soon, the curd being digested finally.

Potato cultures are dry, whitish then yellow, and finally deep orange.

Habitat.—Widely distributed; found in dirty water, sewage, air, dust of streets and houses; also upon the skin; normally present in the mouth, nose, rectum, anterior urethra, vagina, and external ears.

Pathogenesis.—In man it is the cause of carbuncles, abscesses, osteomyelitis, septicæmia, puerperal infection, and any inflammation of the serous membranes. It causes acne and boils; can, and does attack any tissue of the body. Endocarditis is a very grave affection that is caused by this organism. It also plays an important rôle in secondary infection, causing necrosis of previously infected tissues (tubercles) and is active in small-pox and diphtheria. Experimental endocarditis has been produced in animals by injecting it into the veins. By passage through animals it is rendered highly virulent. In young, diabetic and anæmic subjects, its action is often rapidly fatal. Its pathogenic action is often wide and disastrous. By growing it under an aerobic conditions its virulence may be intensified, and the activity with which it liquefies gelatine is an index of its malignancy.

In man acne, boils, and carbuncles have followed the rubbing of culture into the skin.

Immunity.—Careful injections may result in the immunization of the lower animals. An anti-serum with opsonic, agglutinative, lytic and anti-toxic properties has been produced and, if used fresh, seems to have a slight beneficial effect upon staphylococcus septicemia. Too little is known for definite statements. Bacterins made from this germ have been used with excellent results in all but the very aggravated and fulminating affections caused by it. Bacterin treatment of acne and furunculosis has established itself as most efficacious.

There is a member of this group infesting the deep layers of the skin called *Micro. epidermidis albus*. It is of feeble pathogenic power, but may delay the healing of surgical wounds.

GONOCOCCUS

Micrococcus Gonorrhœæ (Neisser).

Diplococcus Gonorrhœæ, commonly called the gonococcus (Fig. 41).

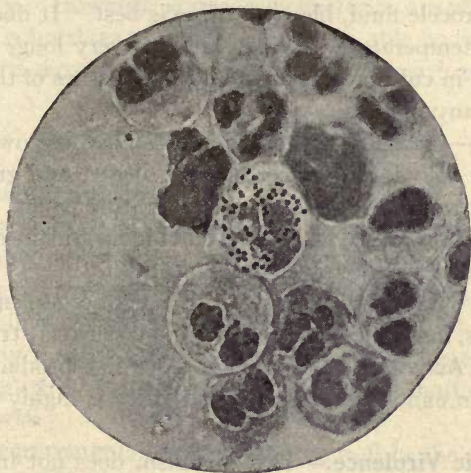


FIG. 41.—Gonococci and pus cells. $\times 1000$. (MacNeal.)

Morphology and Stains.—The morphology of this organism is peculiar and characteristic. Always found in pairs which are cemented by an invisible substance. These pairs resemble coffee beans with the concave sides opposite each other and slightly apart; or kidneys placed with the hilums facing each other.

In pus it is generally found within the protoplasm of the leucocytes, about, though never within, the nuclei. It is non-motile; has no flagella, or spores, and stains readily with all the basic stains; but best with Löffler's blue. It is decolorized by Gram's stain.

This point is most important in differentiating it from other diplococci, except the meningococcus. A diplococcus is said to exist normally in some urethras that resembles the gonococcus, but is Gram-positive.

Oxygen Requirements.—It is a facultative anaerobe.

Vital Conditions.—It is cultivated with difficulty in culture media. Grows best at about 37°C. As it dies quickly in usual culture media, a special one must be employed; that containing ascitic or hydrocele fluid, blood or urine is best. It does not withstand high temperature, drying, or light, very long, and is very easily killed in culture by silver salts. In tissues of the urethra it may live many months.

Cultures.—On agar, containing ascites fluid, it grows very sparingly. The colonies are exceedingly delicate, and gray, turning to yellowish, and are scarcely above the culture media. It will not grow in **gelatine, milk, or ordinary bouillon**, but in one made of nutrose, serum, beef-extract, and peptone.

Habitat.—Never found outside the human organism, except on linen, towels, instruments, etc. It is in all senses a strict parasite.

Bacterial Activities.—Apparently does not produce a soluble toxin, but an endo-toxin (*gonotoxin*), which is highly resistant to heat.

Pathogenic Virulence.—This organism does not infect any of the lower animals. The “gonotoxin,” if injected into small animals, produces a doughy infiltrated area, which undergoes necrosis. It has been found that filtrates of old cultures (sterile), if placed on urethral mucous membranes, can produce suppuration. In man, the organism causes a distressing disease (gonorrhœa), which may become a dangerous one, ending even in death. It may produce violent inflammation of the urethra vagina, uterus fallopian tubes, and the peritoneum. It frequently affects the conjunctivæ, and sometimes causes a pan-ophthalmia, which destroys the sight. It may be a cause of plastic arthritis gonorrhœal rheumatism, endocarditis, pleuritis. In fact, any serous membranes may be infected,

and very serious results follow. Cystitis caused by the gonococcus is sometimes followed by infection of the kidneys. In the urethra, the cocci may burrow deep beneath the epithelial cells, and set up a metaplasia, or abscess formation. The purulent exudate is rich in phagocytes gorged with cocci, often as many as 40 being found within a cell.

Immunity.—One infection does not confer immunity against further infection. There is no reliable means of producing artificial immunity. However, gonococcus bacterins are of some value for chronic gonorrhœa. Torrey has been able to obtain from rabbits an anti-serum of therapeutic value in gonorrhœal arthritis.

MICROCOCCUS TETRAGENUS

Micrococcus Tetragenus.

Morphology and Stains.—Round or oval cocci; found in pairs; more commonly in fours differing in size. In culture this form of growth is apt to vary, and not to be characteristic. In sections of human or animal tissues, tetrads only are found that are always surrounded by a capsule which is stained easily by eosin. The cocci are stained by Gram's method. It is not motile, and does not form spores.

Oxygen Requirements.—It grows very well in the presence of oxygen, and poorly without it.

Cultures.—Grows well on all common culture media. On **gelatine plates** its growth is characterized by small white colonies, elevated, with sharp outlines. It does not liquefy the gelatine. On **agar** it grows even more luxuriantly than on gelatine. In **bouillon** it thrives well, depositing a heavy precipitate. In **milk** it causes acidity but no coagulation. On **potato** it also grows, leaving a silvery streak where the inoculating needle was drawn.

Chemical Activities.—It produces acid in sugar bouillon, but does not form gas, indol, or H_2S .

Habitat.—Has never been found outside the human body; is

normally present in the saliva, sputum of tuberculous subjects, in the cavities of phthisical lungs, and in abscesses.

Pathogenesis.—While causing a fatal septicæmia in mice, and abscesses in rabbits, it is not of much moment from a pathological standpoint, though it plays an important rôle in secondary infection in phthisis and bronchiectasis.

COCCUS OF MALTA FEVER

Micrococcus Melitensis.

Bacterium Melitensis.

Bacillus of Malta Fever.

Coccus of Malta Fever.

An organism belonging somewhere between the Coccacæ and Bacteriacæ. It is small, oval-shaped, and of about $.5\mu$ diameter, occurring in culture singly, in pairs, or in chains. In the latter form, the organism elongates and resembles, more strongly, bacilli. It is non-motile and it has no spores. Stain faintly with the common basic dyes, but not by Gram's method. It has been found in the blood during life, and by splenic puncture.

Cultures.—On gelatine its growth is slow, without liquefaction. On agar the growth, at $37^{\circ}\text{C}.$, is more rapid. The colonies are pearly white, becoming yellow. In bouillon it produces turbidity, with a flocculent deposit. No pellicle is formed. On potato an invisible growth occurs. Milk is not coagulated, nor are acids or gases produced.

Pathogenesis.—It causes in man, Malta fever. Rabbits, guinea pigs, and mice are not susceptible to inoculation, but the disease can be produced in monkeys.

Agglutination.—The serum from an individual suffering from Malta fever agglutinates the bacilli, even in dilutions as high as 1-100.

Diagnosis of the disease can be effected by the agglutination test, and by splenic puncture, and blood cultures.

It is present in the blood and is excreted via the urine and milk. The goat while not suffering with Malta fever can carry the germs in its body and excrete them in the milk. Goats' milk is a general food in Malta. The inference is obvious. Flies may transmit the bacilli.

INFLUENZA BACILLUS

Bacterium Influenzæ.

Influenza bacillus.

Morphology and Stains.—Very small short rods which are often in pairs, found within epithelial and pus cells, and in sputum; from 40 to 80 in a cell. May grow out into short mycelia. No flagella or spores are formed. Stains weakly. Carbol-fuchsin, diluted, gives the best result. The ends of the bacillus stain more deeply than do the rest of the cell. It is decolorized by Gram's stain.

Oxygen Requirements.—It is a strict aerobe.

Cultures grow best on **blood-smear agar**, or in **blood bouillon** between 27° and 41°C.; best at 37°C. Blood or hæmoglobin is demanded for all cultures. In **bouillon** it grows in thin white flocculi. On **agar** in small transparent "dewdrop" colonies, never luxuriantly. Grown in the same culture with *Staphylococcus aureus*, it increases more luxuriantly (symbiosis). It is probable that the cocci, in some way, alter the blood of the culture media. Very satisfactory media may be made by heating blood agar or by the addition of sodium oleate to it.

Vitality.—It is easily killed by light, heat and drying. Lives but a day in distilled water, and from eight to twenty-four hours in dried sputum.

Habitat.—Never outside the body; always a strict parasite. It is found in the mucous membranes of the upper respiratory tract, and in the mucous secretions.

Pathogenesis.—Catarrhal symptoms follow the smearing of a culture upon the nasal mucosa of monkeys. Pure cultures, injected into the peritoneum of guinea pigs cause fatal peritonitis. This bacillus was isolated by Pfeiffer during the influenza epidemic of 1889 and by him believed to be the cause of the disease. Between that time and the pandemic of 1918 it has been found in acute respiratory infections, chronic bronchitis, sinusitis, otitis and meningitis, all attacks being characterized by great depression. Conviction has never been obtained that it was the principal cause of acute disease except for meningitis, but the bronchitis of tuberculosis has been ascribed to it on many occasions. The pandemic cases of 1918 showed a high percentage of positive findings in the sputum and lungs at autopsy. There was however no definite increase in agglutinins, lysins or complement fixing anti-bodies so that many have doubted its etiological relation in epidemic influenza. It was present in a large percentage of cases and certainly aggravated pneumonitis and sinusitis in association with streptococci. Its effect seems due to an endotoxin, possibly also to some exotoxin, having a definite affinity for the nervous system. It circulates in the blood rarely, principally in the meningitic form and in early stages of the intense general cases.

Influenzal meningitis is more frequent than formerly or at least is more often diagnosed. It can be reproduced in monkeys.

By immunizing a goat with influenza bacilli Wollstein obtained a serum which has a pronouncedly favorable effect upon the experimental disease in monkeys and promises some therapeutic power for human beings. Its most important effect is to stimulate phagocytosis in the cerebro-spinal fluid.

A short immunity remains after a spontaneous attack in man but attempts at production of immunity by vaccines have been disappointing. By the use of a mixed vaccine of influenza bacilli, streptococci and pneumococci, the chance of pneumonic complications seems reduced, but influenza may not be prevented.

Therapeutic use of vaccines is useful only to increase leucocytes which are characteristically low during the disease.

Bordet-Gengou Bacillus of Whooping Cough.—This is a very minute ovoid rod lying separately, varying from $.8-1.5\mu$ long and being $.3\mu$ wide. No spores, no motility or flagella. Stains poorly, best at ends; Gram-negative. It may be cultivated from expectoration early in the disease upon media containing glycerine, potato, blood and agar. Aerobe, and grows best at 37°C . There is an endo-toxin. Infective for monkeys. The discoverers claim this to be the cause of pertussis, because it will act as an antigen and fix complement away from the hemolytic series.

Its relation to pertussis has been explained by the report that it is found lying between the cilia of the respiratory epithelium, an embarrassment of the movements of which causes the coughing attack. The only bacteriological diagnosis is by growing the microbes on the medium given above, and by the complement fixation test. Vaccines are said to have a prophylactic value, and some relief of paroxysms certainly seems to follow their use.

The last two microorganisms are types of the so-called hemoglobinophilic bacteria because of the requirement of blood coloring matter in laboratory culture media. Other forms have been found in trachoma and in the spinal fluid.

Conjunctivitis.—There are two specific germs for conjunctivitis separate from the gonococcus. They are the bacillus of *Koch-Weeks* and that of *Morax and Axenfeld*.

Koch-Weeks Bacillus.—The organism of pink eye. This is a minute, $1.5\mu \times .2\mu$ non-motile, Gram-negative, sporeless, poorly staining rod, very like the influenza bacillus. It is aerobic and non-liquefying. It grows as minute, pearly, glistening, discrete colonies only upon agar of 5 percent strength plus serum.

The Bacillus of Morax and Axenfeld.—A non-motile, sporeless *diplo*-rod; negative to Gram stain. Grows only in the presence of serum or blood and liquefies the former. It is larger than the Koch-Weeks bacillus, measuring up to 2μ .

PLAGUE BACILLUS

Bacterium Pestis.

Plague Bacillus (Fig. 42).

Morphology and Stains.—Short plump rods with rounded ends, containing no spores and non-motile. It is said by some that a capsule is formed. Organisms from exudates, or blood, exhibit characteristically peculiar polar staining. They are often found

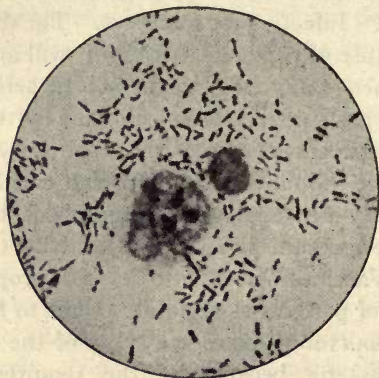


FIG. 42.—Pest Bacilli from spleen of rat. (Kolle and Wassermann.)

within the leucocytes. In bouillon the organism grows in long chains; is stained with all the common basic dyes, but is not colored by Gram's method in cultures. It exhibits a great variety of involution forms when grown in salty culture media ($3\frac{1}{2}$ percent salt).

Relation to Oxygen.—Strict aerobe, the growth is stopped by the exclusion of oxygen.

Vital Requirements.—Grows well at $22^{\circ}\text{C}.$, but best at $35^{\circ}\text{C}.$; is killed after a short exposure to $55^{\circ}\text{--}60^{\circ}\text{C}.$, stands drying from four to eight days, and dies in water after a week. In the buried bodies of man and animals it lives from twenty-two to thirty-eight days. Withstands freezing for months, but does not stand light or chemicals very long.

Cultures.—Grows very well on culture media. In **bouillon** it thrives abundantly, with a heavy pellicle which produces dependent stalactites that drop to the bottom of the vessel. On **gelatine plates** it grows in small flat colonies, which are gray and transparent, and which do not liquefy the gelatine (Fig. 43). In **gelatine tubes** it forms a faint thread-like line, without liquefying the media. On **agar** the growth is whitish and abundant, and resembles the colon bacillus. Old cultures are luxuriant. **Milk**

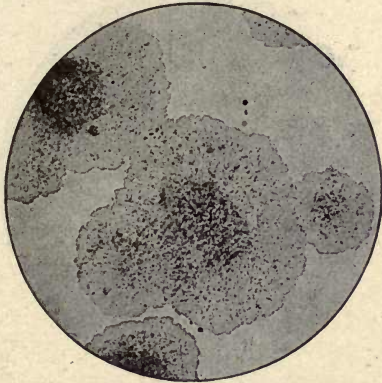


FIG. 43.—Colonies of plague bacilli forty-eight hours old. (Kolle and Wassermann.)

is not coagulated, but a faint acidity appears. **Potato** yields a slow whitish-yellow growth that is sharply outlined.

Chemical Activities.—Does not produce H_2S , enzyme, colors, or odors, indol or nitrites. The toxin produced is not soluble and the filtrate is non-poisonous. Old killed bouillon cultures can be extracted and a highly poisonous substance precipitated therefrom with alcohol, or ammonium sulphate, that is lethal for mice.

Habitat.—Never found in healthy human bodies. In persons afflicted with plague, the organism is widely distributed in buboes and in the cutaneous pustules, lymphatics and in the lungs in plague pneumonia; more rarely in the blood and other organs.

In **animals**, plague occurs in rats. It is supposed that some tropical soil bacilli infect rats, and becoming accustomed to the rodent's body, are eventually transmitted to man. The bacilli may be transmitted from rat to rat in India by the rat fleas which also can bite man. The organisms remain in the flea for some time. Rats are also infected from dead rats. In epidemic times the soil becomes infected and persons going barefoot may be infected.



FIG. 44.—*B. Pestis* in pus of bubo. (Jackson.)

Pathogenesis.—Highly pathogenic for man. Is the cause of the bubonic or Oriental plague; bacilli gain entrance by way of the skin, causing localized foci of infection from which buboes develop, followed by pest-sepsis and death. The lungs may be the original site of invasion, and plague pneumonia (worst form of the disease) may result. The typical bacilli can be found in the sputum of the patient thus affected but not in quietly expired air. The mortality from this plague is from 50 percent to 80 percent.

Almost all domestic animals—rats, mice, guinea pigs, rabbits and squirrels are susceptible; horses and swine are very susceptible; cows and dogs less so. Rats seem to be affected with a

chronic form of the malady, and by inhabiting ships and warehouses in foreign countries, spread the disease. Post mortems on infected animals reveal hæmorrhagic petechia and serous infiltration into serous cavities. Death is generally due to a profound toxæmia and exhaustion.

The virulence of the organism can be raised by passing it through a series of animals.

Serum from infected animals agglutinates plague bacilli.

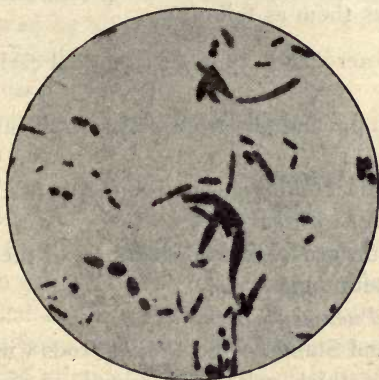


FIG. 45.—Pest bacillus involution forms produced by growing on 3 percent salt agar. (Kolle and Wassermann.)

The diagnosis of the plague bacilli is made by rubbing the suspected culture upon the freshly shaven skin of a guinea pig; if the animal develops buboes and dies, and polar staining bacilli are found, it is probable that the organism is the plague bacillus. Further, if curious involution forms develop on heavily salted agar (3 percent) the diagnosis is confirmed (Fig. 45).

Immunity.—It is possible to immunize against the disease. Kitasato and Yersin produced an anti-toxic serum, which has, not only a prophylactic, but a curative action. By the use of killed culture Haffkine vaccinated many people against the plague very successfully (see page 83).

MUCOSUS CAPSULATUS GROUP

There is a large group of organisms of moderate pathogenic powers and importance called variously, *Bacterium aerogenes*, *Bacterium mucosus* or *Aerogenes mucosus* group of which the Friedländer bacillus is the most important. They all have a luxuriant growth on media; are negative to Gram stain; ferment most of the carbohydrates; are non-motile and most of them show a capsule when in the animal body.

Perkins divides them as follows:

I. *Bacterium aerogenes* type ferments all carbohydrates with gas.

II. *Bacterium pneumoniae* group ferment all carbohydrates but lactose, with gas.

III. *Bacterium lactis aerogenes* group ferment all carbohydrates except saccharose, with gas.

These organisms are important members of the intestinal flora.

Bacterium Pneumoniæ.

Friedländer's Pneumonia Bacillus.

Morphology and Stains.—Short plump rods with rounded ends, surrounded by a thick gelatinous capsule in animal fluids, and when grown in milk; is not motile, and has no spores; does not stain by Gram's method, but easily by the common basic dyes.

Oxygen Requirements.—Grows in and without oxygen, upon all culture media.

Chemical Activities.—Produces abundant acids, CO_2 and H_2 , gas, alcohol, indol, ferment and H_2S .

Habitat.—Has been found in soil; sometimes in healthy saliva.

Culture Media.—Grows luxuriantly on all culture media.

On gelatine it grows in roundish elevated colonies that are yellowish-white with a slimy lustre, and never liquefies the gelatine. In agar it multiplies even more abundantly with a moister growth. The border of streak cultures is smooth and wavy, and the water of condensation is cloudy. In bouillon the growth is

very cloudy with a silvery deposit at the bottom. The bouillon becomes thickened. **Milk** is not coagulated, and **potato** yields a luxuriant yellowish, moist shining growth.

Pathogenesis.—It is possible to cause pneumonia in mice, also septicæmia. Guinea pigs and dogs are susceptible. It may be found in normal mouths. Friedländer's pneumonia is much less frequent than that due to the pneumococcus, but it is very fatal. Members of this group may also be responsible for cystitis, pyelitis, sinusitis and in children pneumonia and pleuritis. The exudate produced by all of them is mucoid, stringy. Anti-bodies are not readily formed against any of the mucosum group so that anti-sera and vaccines are not of great value.

The *Bacterium lactis aerogenes* group is a very large one and includes nearly all the forms engaged in milk souring. The ordinary *B. lactici* is very like the colon bacillus, but is non-motile. It forms lactic acid among its principal products. The most important lactic acid producer related to but not belonging directly in this group, is *Bact. bulgaricum* of Massol. This is the principal ferment of the eastern sour milks, Kumyss and Yoghurt. Because of the large amount of lactic acid formed by this germ, Metchnikoff has advocated cultures of it and sour milk made by it in the treatment of intestinal putrefaction and fermentation. The *Bacterium bulgaricum* produces a soft milk curd and an excess of lactic acid and alcohol. The bacteria are non-motile, non-spore-forming, Gram-positive and vary from 2μ to 50μ in length. They grow with difficulty in the laboratory, best on milk and whey. Optimum temperature 44°C . They form branching filamentous colonies. Milk is coagulated in eighteen hours at 44°C . and in thirty-six hours at 37°C . The clot is not dissolved. Gelatine is not liquefied. Congeners with this organism are *Bac. acidophilus* and *Bac. acidophil-aerogenes* differing from it in fermentative powers.

TYPHOID BACILLUS

Bacterium Typhi. Eberth.

Bacillus Typhosus

Typhoid Bacillus (Fig. 46).

A most important pathogenic organism which causes typhoid fever.

Morphology and Stains.—Generally short plump rods 1 to 3μ long, and .6 to $.8\mu$ broad. Forms long threads in cultures, especially on potatoes. Polar metachromatic bodies are sometimes seen as are unstained areas when alkaline methylene blue is used. The rod is flagellated (peritrichous); contains no spores; exhibits pleomorphic and involution forms; is actively motile, and stains with all the basic aniline dyes, but not by Gram's method.



FIG. 46.—Typhoid bacilli showing flagella. (Kolle and Wassermann.)

Vital Resistance.—The thermal death-point is $60^{\circ}\text{C}.$, ten to fifteen minutes. Remains alive in ice for three months; even the temperature of liquid air does not destroy it at once. In distilled water it lives for months, but if other saprophytic bacteria are associated with it, however, it quickly dies. Does not resist drying or chemicals, except carbolic acid, towards which it exhibits a tolerance. Sunlight kills it in an hour.

Habitat.—It never exists in nature, except where water or soil has been contaminated by fæces or urine. It may multiply in potable waters, in milk, and the juices of oysters.

Chemical Activities.—Does not produce proteolytic enzymes; forms H_2S , but will not ferment the sugars with gas formation. Does not yield indol or nitrites. Produces levorotatory lactic acid. Its toxin is all contained within the bacterial cell (endotoxins) and is not water-soluble. This toxin is manifested by injecting washed and killed bacilli into animals, or by freezing the

bacilli with liquid air, and then crushing them. This injected into guinea pigs causes diarrhœa, mydriasis and death.

Oxygen Requirements.—It is a facultative aerobe.

Cultural Characteristics.—It grows upon all media at the temperature of the body, 37°C. and more slowly at 20°C. On **gelatine plate** it produces at first small colonies, yellowish and punctate, which become whitish, delicately notched and ridged (Fig. 47). In **gelatine stab** culture it grows in a thread-like granular

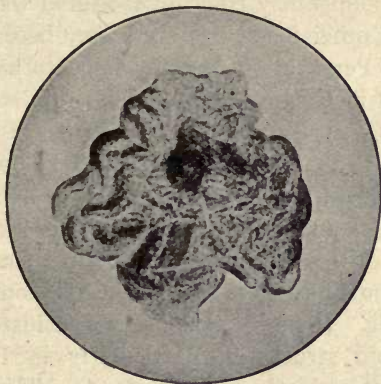


FIG. 47.—Seventy-two hour old culture of typhoid bacillus on gelatine. (Kolle and Wassermann.)

line, without producing gas. In neither case is the gelatine liquefied. On **agar plates** the colonies are not so characteristic, being round, grayish-white, and shining. In **milk** it grows well, not coagulating it even after boiling, and only a very little acid is produced. On acid **potato** the growth is characterized by its invisibility, and this fact is used to differentiate it from other kindred bacteria. The growth is detected only by scratching with a needle. In **bouillon** it grows uniformly, producing very little acid, and no gas. In special media (Hiss's semi-solid media) thread-like colonies are produced, which are characteristic. On Elsner's potato media it produces small granular, glistening

points. It also grows characteristically in Endo and the Drigalski and Conradi media. In sugar media no gas is formed but there is acidification in dextrose, galactose, mannit, maltose, xylose and levulose. The addition of sterile bile to culture medium, 10-50 percent, increases the chance of isolating the germ in blood, fæces or urine cultures; it acts by inhibiting other organisms and by supplying salts favorable to the typhoid bacillus.

Invasion of Body.—This organism generally invades the body by way of the alimentary tract, in food and water. Flies may infect milk and other foods. Oysters may become infected and cause disease. Personal contact, by hand to hand for example, is a very potent method of transmission.

Pathogenesis.—It is certainly the cause of typhoid fever. During the attack the germs circulate in the blood-stream during the entire fastigium but are obtained with ease only in the first two weeks. They are constantly in the fæces for varying periods even after clinical recovery and may be frequently found in the urine. Rose spots also contain them. Also found in the spleen and gall-bladder. It produces well-marked histological changes in the lymphoid structures, particularly in Peyer's patches, solitary follicles, and other lymph-glands. There is, according to Mallory, a massive endothelial proliferation in the lymph-glands. This causes occlusion of the lymph-vessels, and is followed by necrosis (ulceration) of the Peyer's patches. The intense phagocytic action of the fixed lymphatic cells in the glands is manifest toward the red blood cells, which are devoured in great numbers. The toxin causes degeneration of other organs, particularly in the liver. Bacilli are found in the spleen and blood. The rose-colored spots are found to be full of them. The disease is certainly not a merely localized infection of the lymph structures, but is a bacteriaemia. There is often a mixed infection in which streptococcus pyogenes in the blood plays an active rôle. In the necrosis of bone and in subphrenic abscess the typhoid bacilli may act as a pus former. Commonly it

produces death by (1) profound toxæmia; (2) ulceration of the Peyer's patches, causing perforation and peritonitis; (3) by the destruction of a blood-vessel in the floor of an ulcer producing a hæmorrhage.

In animals, as a rule, typhoid bacilli if injected, produce no disease, and the bacilli rapidly die. In chimpanzees, however, it is possible to produce atypical typhoid lesions and symptoms.

Natural and Acquired Immunity.—Human blood serum is strongly bactericidal toward the typhoid bacillus. Normal gastric juice, with its hydrochloric acid, destroys the bacillus when ingested and this forms the natural means of protection. Immunity following an attack of typhoid is generally of long duration. If bacilli do reach the blood-stream of an immune individual, the amoebocytes originated by a previous infection, together with the complement normally present, effect a solution of the invading organism. For vaccination against typhoid fever see page 80. Anti-serum for typhoid has been prepared by injecting horses with killed culture of typhoid bacilli, but it has not proved to be effective.



FIG. 48.—Widal reaction. One-half of the field shows typhoid bacilli unclumped, other half shows clumping. (Greene's Medical Diagnosis.)

Agglutinations.—One of the most important means of diagnosing typhoid fever is by the so-called Widal test, really the Gruber and Durham agglutination reaction. This consists in applying the serum of the blood of a person, supposedly ill with typhoid, to a fresh bouillon culture of typhoid bacilli. If the person has the disease, and it has lasted for five or more days, the bacilli are promptly agglutinated in clumps. Undiluted normal serum, and serum from people suffering other diseases, will bring about the same reaction at times; it is therefore best to dilute the serum with water 1-50, and if the reaction comes within an hour the

disease is considered typhoid fever. The test may be either with a hanging drop and examined microscopically, or macroscopically by adding a drop of diluted serum to fresh bouillon culture of typhoid bacilli, when, if the case is typhoid, large clumps of the bacilli will form and drop to the bottom of the tube. Animals immunized against typhoid exhibit this reaction to a high degree. Serum diluted with 10,000 parts of water has caused the reaction in less than one hour's time. This reaction with a known culture of typhoid bacilli is used clinically to identify serum from a doubtful case of typhoid, and establish a diagnosis. On the other hand, a known serum prepared artificially by immunizing rabbits with bacilli is used to identify typhoid bacilli when found in water, or elsewhere. There are two stages to the reaction; immediately after mixing the serum and culture, the bacilli will be seen to become less motile, and then still. After this they begin to huddle together into clumps. In complete reaction they remain immobile and tightly massed. In some cases bacteriolysis occurs, and many of the bacteria are dissolved in the serum. The foetus of a woman suffering from typhoid contains agglutinins in its blood. The milk, tears, and other body fluids from an individual with typhoid, agglutinate typhoid bacilli. Serum to perform the test may be obtained by puncturing the skin, or by blistering it and drawing off the serum, or else by abstracting blood from a vein with a hypodermic syringe.

Agglutinin appears during typhoid, generally after the fifth day, and persists for some time (several years?) after convalescence, or if the actual agglutinin titer be not high in an immune person, it rises rapidly should typhoid bacilli gain entrance to the body. In persons vaccinated against the germ, the agglutinin titer remains partly high for at least six months but when the amount shall have returned to normal, it has the power to revive rapidly should infection threaten. Agglutinins are in a measure an index of resistance, or at least their facility of action is our guarantee of protection.

Paratyphoid Bacillus.—A pathogenic organism producing all the clinical symptoms of typhoid, only in milder form (at times) has been discovered. It differs from the true bacillus because it ferments dextrose and maltose producing gas and acid, and is not agglutinated by the serum from a true typhoidal infection. There are several closely related varieties differing in growth upon litmus milk and in fermenting several sugars but in other respects they resemble the typhoid bacillus, and seem to occupy a position between it and the colon bacillus. Paratyphoid endotoxin resists 60°C. from thirty to sixty minutes and in the case of the organisms of meat poisoning, paratyphoid beta, paracolon and the Gärtner bacillus, a short exposure to the boiling point does not seem to destroy the toxin.

It is generally taught today that the foregoing organisms produce infections of similar clinical characters in that they are contracted in the same manner, have comparable pathology and immunity reactions and are amenable to the same prophylactic measures; they are designated "the typhoid fevers."

The Paracolons are organisms like the paratyphoids, but somewhat closer to the colon bacillus (for example, see page 177). This term is best applied to organisms of the meat poisoning group, as the Gärtner bacillus, the hog cholera bacillus, so that the varieties which cause typhoid fever in man can be recognized under the term paratyphoid.

Blood cultures are often employed in large hospitals for the diagnosis of typhoid fever. During the first week of the attack bacilli may be recovered from the blood by withdrawing 10 c.c. of blood from a vein and mixing it with 500 c.c. of bouillon. The large amount of blood is necessary, because the bacilli are few in number, and the bactericidal action of the serum outside the body is powerful until mixed with the bouillon, after which the bacilli are able to withstand it. The bacilli may be easily isolated from the blood by adding the latter to some bile and then incubating it. From the bile, cultures are made in agar or in bouillon.

COLON BACILLUS

Bacterium Coli.

Bacillus coli or *Bacillus coli communis*.

Colon Bacillus.

While not strictly a pathogenic organism, it plays such an important part in secondary infection, and resembles so closely the typhoid bacillus, that it will be described here.

Morphology and Stains.—Is not so motile as typhoid; has not so many flagella; and is devoid of spores. It exhibits pleomorphism; may grow in chains; and possesses vacuoles and polar

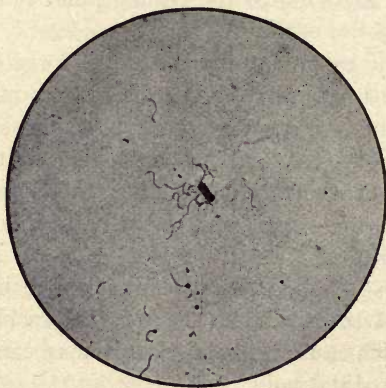


FIG. 49.—Colon bacillus showing flagella. (Kolle and Wassermann.)

bodies at times. Is readily stained by all the common basic stains, but not by Gram's method.

Oxygen Requirements.—It grows especially well in oxygen; without oxygen its growth is not so good.

Temperature requirements, and vital resistance. It grows well at room and incubator temperature. Its thermal death-point is about 62°C.; light and heat are destructive to it, and its resistance to antiseptics is somewhat less than that of typhoid bacillus.

Cultures.—Thrives in all common culture media, especially if sugar is present. It is restrained by excess of acids produced in culture media. On **gelatine** it grows like the typhoid bacillus (from which it is difficult to differentiate, see page 176) in whitish raised colonies that do not liquefy the media. Sometimes the growth is thin and iridescent, and exhibits bizarre shapes—tadpole-like and lobulated. Typhoid colonies show deep furrow-like ridges under the microscope. In the special semi-solid media of Hiss, the typhoid produces uniform cloudiness, with thread-like colonies. The colon does not so quickly cause this cloudiness, and forms gas bubbles. On **agar plates** surface colonies are like typhoid, only they are thicker and moister. If litmus is added to this medium, a red zone forms about the colonies due to the presence of lactic acid. In **agar tubes** the growth is more luxuriant and resembles typhoid. In **litmus bouillon** it rapidly reddens the litmus, clouds the medium, and deposits a slimy sediment. In **milk** it always produces coagulation. On **potato** it grows more rapidly and luxuriantly than typhoid, at first yellowish-white, which later changes to yellowish-brown. It is slimy.

Chemical Activities.—Produces color on potato only. Sugars are fermented with the production of H_2 , CO_2 and some N_2 . It ferments glucose, lactose, saccharose, maltose, dulcitol and some others with the production of gas. Produces lactic, acetic and formic acids, also indol abundantly, and H_2S . It decomposes urea.

Habitat.—Found always in the intestinal contents of most animals and man. Also in streams and rivers that run through farm lands and by towns. While it is difficult to find typhoid bacilli in contaminated drinking water, the colon bacilli are easily found. If in abundance, it indicates great faecal pollution. In milk it is often found, where it plays an important part in souring.

Pathogenesis.—It is pathogenic to rabbits and guinea pigs, causing peritonitis if injected into the peritoneal cavity. In man

it plays rather a subordinate pathogenic rôle, but it has been found the causal agent of some cases of suppurative appendicitis, peritonitis, and cystitis. It may attack the lungs and meninges of feeble children, and cause death by setting up a pneumonia or meningitis. During the agonal period in wasting diseases it may cause terminal infection and hasten death. Colon bacilli encysted in the liver and kidney have been found by Adami in cirrhosis of these organs, and it is believed by him to be partly the cause of these diseases; chronic infections of the rectum are due to this organism.

Agglutination.—Animals immunized against colon bacilli by repeated injections, exhibit agglutinins in their blood.

The differentiation of the typhoid from the colon bacillus is largely accomplished by noting the chemical reactions of both organisms in culture media. The chief differences are:

1. The typhoid bacillus has more flagella than the colon, and is much more motile.

2. On gelatine culture plates, the typhoid colonies develop more slowly than the colon, and are much more delicate and transparent. If litmus is present the colon colonies are red, the typhoid bluish.

3. In media containing dextrose, or lactose, gas is produced by the colon, but not by typhoid.

4. In peptone solution the colon produces indol, while the typhoid does not.

5. Milk is coagulated by the colon, but not by the typhoid.

6. On potatoes colon grows much more luxuriantly than typhoid.

7. Typhoid reddens neutral red; colon changes it to bright yellow.

8. The most important test is the agglutinative one. Typhoid is clumped by anti-typhoid sera, highly diluted, while the colon is not.

No anti-sera of value have been found for colon bacillus infection, but bacterins have been used with much benefit.

GÄRTNER'S BACILLUS

Bacillus Enteritidis.*Bacillus of Gärtner.*

The cause of one form of meat poisoning, and closely allied to the paratyphoid bacillus in its morphological characteristics. It gives a classical picture of the type "paracolon."

Morphology and Stains.—This organism is a short plump ovoid; is motile; has about eight flagella; does not form spores; and stains well with all the common aniline dyes, but not with Gram's method.

Vital Resistance.—It is a facultative anaerobe. It is destroyed by means outlined for the colon bacillus when in culture. In meat it must be subjected to prolonged heating.

Cultures.—Grows on all the common culture media. In **bouillon** thrives well, producing gas in media containing dextrose. It ferments without gas production **lactose**, galactose, maltose, and cane sugar. Does not produce indol, which distinguishes it from the colon bacillus, to which it is closely allied. In **milk** it reduces litmus and coagulates the casein in a few days. On **potato** it grows well, producing a yellowish shining layer. On **gelatine** it multiplies without liquefying the medium. Superficial colonies in plates are pale and gray, deep colonies yellow and spherical.

Chemical Activities.—Acid, gas and a powerful heat-resisting toxin which is soluble, are found. Infected meat contains this toxin, which is not destroyed by cooking.

Pathogenesis.—It is pathogenic for man, horses, cattle, and laboratory animals. Neither the bacilli nor the toxin they elaborate are destroyed by moderate heat. Flesh is infected before death, after which, both the bacilli and toxin increase. Mischief follows the partaking (usually in the form of sausages, etc.) of this meat, causing, in men, violent nausea and diarrhoea, skin eruption, and in severe cases, pneumonia, nephritis, collapse and

death. Mortality is from 2 percent to 15 percent. The post-mortem findings are not specific. There may be evidence of an enteritis with swollen lymph follicles, and an enlarged spleen.

Agglutination.—The blood of infected individuals may agglutinate bacilli. A dilution of such blood with 8,000 parts of water has produced the reaction.

No anti-serum or bacterin treatment is as yet possible.

DYSENTERY BACILLUS

Bacterium Dysenteriae.

Dysentery Bacillus.

The cause of one form of tropical dysentery. The group to which this belongs comprises many closely related varieties some of which are thought to be the cause of infant diarrhoea in this country. There are numerous varieties of this organism, the differentiation of which depend upon their chemical activities, fermentation of various carbohydrates being the most important, and agglutinative properties with different sera. The tropical form of dysentery is due to the type originally described by Shiga; this type is uncommon in temperate zones, the Flexner variety being much more common. The Shiga variety is much the more virulent.

Morphology and Stains.—The organism is, in many respects, similar to the typhoid bacillus, but is plumper. It is non-motile, has no spores, and exhibits pleomorphism. It stains well with the common aniline dyes, but not by Gram's method.

Vital Properties.—It is killed by 1 percent carbolic solution in thirty minutes. Lives for twelve to seventeen days when dried. Direct sunlight kills it in thirty minutes. Its thermal death-point is 58°C. in thirty minutes. It is a facultative aerobe; grows at ordinary temperature, but better at 37°C.

Cultures.—Grows on all the common culture media, but more slowly than the colon bacilli. **Gelatine** cultures resemble typhoid. The growth in this media (which it does not liquefy) produces no

pellicle, but a sediment. Indol is not produced, and **milk** is first mildly acid and then faintly alkaline, though not coagulated. On **potato** it grows sparingly, often turning it brown. The Shiga type ferments glucose, but no other sugar. The Flexner type ferments glucose, dextrine, and mannite, but not lactose. The latter type produces more acid than the former, and both are best agglutinated with their corresponding serums.

Habitat.—In living bodies the organism is found solely in mucous discharges from the bowels. In the dead it is found in the lymph-glands. If it reaches the circulation, it appears to be rapidly destroyed by the blood. It has been discovered, however, in the body of a fœtus delivered from a woman with the disease. The organism must have passed the placenta of the mother. The disease is spread by water, food and personal contact and by carriers, and it may become epidemic in large institutions.

Pathogenesis.—The typical lesions caused by the organism vary from a mere hyperæmia to a superficial necrosis of the lymphoid structures, which may be extensive. Peyer's patches are slightly swollen but not ulcerated. The descending colon and sigmoid are oftenest attacked. The necrotic masses separate, leaving shallow ulcers. The lymph structures are engorged with polynuclear leucocytes. No marked lesion is found in the spleen. The liver and kidneys often undergo marked parenchymatous degeneration. The bacilli being possessed of a powerful endotoxin, so that dead cultures, if injected under the skin cause marked local and general reactions. Like the pyocyaneus bacillus, this organism undergoes auto-digestion in bouillon, which leaves the latter highly toxic owing to the liberation of the toxins. Laboratory animals quickly succumb to injection of this organism, injection producing a marked reaction in the colon, a phenomenon suggesting that there is a predilection for the organ and that the body uses it as an excretory organ for the poison. Dysentery cannot be induced in animals by feeding cultures. Poorly nourished subjects are easily infected and quickly die. Digestive

disorders favor infection. Death may be due to toxæmia or exhaustion. As a causal agent in the production of summer diarrhoeas of children, the dysentery bacillus plays a part. It has been isolated from the stools of infants, with this disease, and their sera have been found to agglutinate the bacilli. Nevertheless it is known that other bacteria (streptococci, etc.) cause this disease, and Weaver found that "clinically twenty-four of our ninety-seven cases of ileocolitis in which dysentery bacilli were discovered did not differ from cases in which dysentery bacilli were not found.

Immunity.—The sera from convalescents from dysentery show a strong bactericidal action. Anti-bodies are developed by infection and by artificial inoculation with killed cultures. Kruse obtained a serum from horses which strongly protected a guinea pig against a lethal injection of bacilli. The protective property of the serum is due to its bactericidal action. Here the amboceptors act, but only in the presence of a complement. It is possible that a small amount of anti-toxin is present since there is some reason to think that a modicum of free toxins is produced by the bacilli.

Vaccination.—Shiga tried to induce (1) passive and (2) active immunity in many individuals by injecting both anti-toxic serum and bacteria into them. This was not followed by a lowered number of infections, but by a lowered mortality. A serum may be produced by injecting horses with several dysentery strains, called a polyvalent anti-serum. This has good therapeutic effects but does not immunize prophylactically.

Agglutination.—The serum from a patient suffering from either dysentery or summer diarrhoea, will, after about a week's illness, agglutinate bacilli. This property is not always present, and its absence does not exclude the possibility of infection. In performing the reaction, both Shiga's and Flexner's type of organism should be used. These types probably bear the same relation to each other that typhoid and paratyphoid do.

PYOCYANEUS BACILLUS

Bacterium pyocyaneus.

Bacillus Pyocyaneus (Fig. 50).

Bacillus of Blue Pus. Also called *Pseudomonas pyocyanea*.

An organism of minor importance as a pathogenic agent, that is often met with in groin or axilla.

Morphology and Stains.—Slender rods, often growing into thread-like forms. Exhibits pleomorphism. Sometimes is rounded and cocci-like, is motile, has a polar flagellum, and

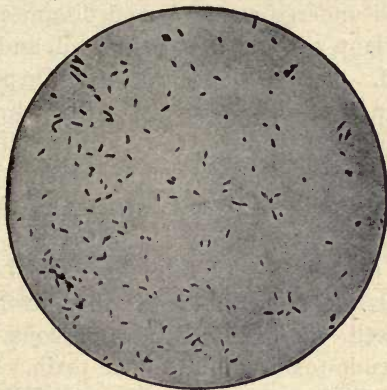


FIG. 50.—*Bacillus pyocyaneus*. ▲ (Kolle and Wassermann.)

no spores. Stains with all the basic aniline dyes, but not with Gram's method.

Oxygen Requirements.—Usually a strict aerobe.

Cultures.—Grows on all the **common culture media** luxuriantly, at room and incubator temperatures. It elaborates two pigments, a water-soluble greenish bacteriofluorescein, and a chloroform soluble pigment, a beautiful blue in color, called pyocyanin. On **gelatine plates** it produces yellowish-white to greenish, yellow colonies which liquefy the **gelatine**, causing crater-like excavations about the colonies. **Gelatine stab** cultures rapidly liquefy along

the line of inoculation, coloring the gelatine greenish-blue, and a white crumbly deposit forms in the bottom of the stab. On **agar plates** it produces yellowish-white colonies, surrounded by a zone of bluish-green fluorescence. It grows luxuriantly. In **agar tubes** it multiplies rapidly, spreading over the medium, with wavy thickened edges. The **agar** quickly turns a dark greenish-blue, and in old cultures the growth changes from yellow to greenish-blue.

In **bouillon** it is very dense and yellowish-green; a pellicle forms on the surface, and a sediment is deposited. In old bouillon cultures the bacilli undergo autolysis and disappear. In **milk** the growth is luxuriant, the casein is coagulated, and the clot is ultimately digested. The reaction is alkaline. On **potato** it varies in luxuriance, often being slightly elevated, yellowish, turning to green. The variance in growth is due to the kind of potato used. Drying kills the organism speedily; four hours in sunlight also destroys it.

Chemical Activities.—No gas is generated. Besides the pigments (already specified) ammonia is produced, also a peculiar enzyme called pyocyanase by Emmerich and Löwe, which not only digests gelatine and milk curd, but its own and other bacterial cells as well. Old cultures are poisonous; a hæmolysin is produced—an endo-toxin, and a soluble toxin. The last-named toxin stands a temperature of 100°C. Against the endo-toxin and the soluble toxin it is possible to prepare an anti-serum. This may protect laboratory animals.

Pathogenesis.—Has been found a sole cause of meningitis and vegetative endocarditis in man; is a pyogenic organism; can cause suppuration anywhere in the body; produces blue pus; is pathogenic to guinea pigs; and its virulence can be raised by passing it through a series of animals.

Agglutination.—The serum of infected and immunized animals both in moderate dilution causes agglutination of bacilli. It is possible to use bacterins of this germ. Bactericidal substances develop by the use of killed cultures.

BACILLUS OF SOFT CHANCER

Bacterium Ulceris Chancrosi (Ducrey).

Streptobacillus of Soft Chancre.

Morphology and Stains.—A small thin bacterium $.5\mu$ broad, 1.5μ long, growing in chains with polar staining, which can be demonstrated in sections of chancroids without much difficulty.

This organism does not stain by Gram's method, but by Löffler's it is stained with ease.

Cultures are hard to make. It grows best in **serum agar**, and **blood agar** in faint colonies that are not very characteristic. In condensation water of agar it grows feebly.

In sections and in pus the organism is frequently found in the interior of leucocytes.

By aspirating pus from buboes and planting it on old but moist blood agar plates, cultures may be obtained.

Pathogenesis.—From an old culture of over ten generations typical ulcerations were produced in man. The organism is feeble and quickly dies in culture media or in contact with mild antiseptics.

ANTHRAX BACILLUS

Bacillus Anthracis.

Anthrax Bacillus of Koch (Fig. 51).

Practically the first pathogenic organism to be isolated. This was accomplished by Dr. Robert Koch. It is the cause of a widespread malignant disease, variously called Anthrax, Charbon, or Splenic Fever. Animals and man are infected by it, and its action is often rapidly fatal.

Morphology and Stains.—In animal tissues this organism appears as a large rod $3-10\mu$ long, and $1-1.2\mu$ wide. Is often in pairs or chains. In fresh specimens the ends of the rods are rounded; when older, the ends become square or concave. Often they have faint capsule surrounding them. In culture media they exhibit

spores and grow in long threads, these threads form long spirally twisted masses, like locks of wavy hair. No flagella are formed, and the organism is not motile. In old cultures, bizarre involution forms are found. It stains well with all the common basic dyes and by Gram's method.



FIG. 51.—Anthrax bacilli in blood. (Greene's Medical Diagnosis.)

Oxygen Requirements.—Is a facultative anaerobe, but grows much better in the presence of oxygen. If oxygen is excluded, no liquefaction occurs.

Temperature.—Grows between 14°C. and 45°C. ; best at 37°C. Spores are formed, if oxygen is present, between 15°C. and 40°C. Sporulation is more rapid at 37°C. Spores withstand high temperature (dry) for a long time, 100°C. for one hour. The bacillus itself is killed at 70°C. , moist heat, in one

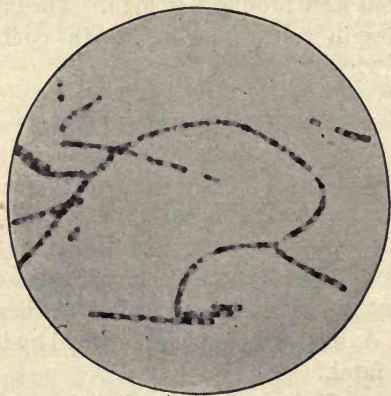


FIG. 52.—Anthrax bacilli growing in a chain and exhibiting spores. (Kolle and Wassermann.)

minute. The thermal death-point may be put down for the organism, at 100°C. steam, for five minutes.

Vital Resistance.—Highly resistant to chemicals, light and drying. Spores resist 5 percent carbolic solution for days (Esmarch), but 1–1000 corrosive sublimate for only a few hours. They also resist formaldehyde and sulphur for a long time, and withstand light. A 2 percent fresh solution of H_2O_2 kills spores in three hours. Three and one-half hours' exposure to bright sunlight killed the spores if oxygen was not excluded (Dieudonne) (Fig. 52).

Sporulation Phenomena.—At $12^{\circ}C$. spores are formed if oxygen is present. The most favorable temperature for sporulation is that of the body ($37^{\circ}C$.). Spores are never found in the bodies of living or dead animals if they remain unopened, and oxygen is excluded. If bacilli are cultivated at $42^{\circ}C$. for a long time and frequently reinoculated, on fresh media, the ability to form spores is lost even if grown again at $30^{\circ}C$. (Phisalix). If cultivated upon media containing carbolic acid and hydrochloric acid, the ability to sporulate may be lost.

Chemical Activities.—Acetic acid is formed, as is H_2S . Liquefying, milk coagulating, and milk digesting enzymes are formed. Toxins have not been isolated, but may be produced.

Habitat.—Only found where infected animals, hides, and hair have been. Fields, hay, bristles, hides, manure, etc., have been found to contain bacilli. Drinking water may be polluted by tanneries and the bodies of dead animals. Meadows and fields may be contaminated for years. From the buried bodies of infected animals anthrax spores may be brought to the top of the soil by earth-worms.

Cultures.—Grows exceedingly well on all culture media in the air. On **gelatine** it grows in whitish round colonies, rapidly sinking into the gelatine, due to the liquefaction. The liquid medium is turbid. The interior of the colony is crumbly. When magnified, the colonies seem to be made up of tangled waving bundles, like locks of hair, especially about the periphery. In **gelatine stab cultures** the growth is luxuriant and rapid; the medium is liquefied more rapidly at the top, and finally a crater is formed; before this

appears, lateral hair-like outgrowths are seen in the gelatine. At the bottom of the crater a white crumbly mass is formed, but no pellicle. On **agar plates**, small whitish colonies develop which are elevated and round. When magnified, wavy hair-like growths appear on the edge, caused by many twisted parallel chains of bacilli (Fig. 53).

In **agar stab**, the growth is more luxuriant near the top; lateral filamentous branches are seen along the stab line. In **agar streak** the colonies are abundant, thick and fatty; have tangled edges, and the water of condensation is cloudy. In **bouillon**, it

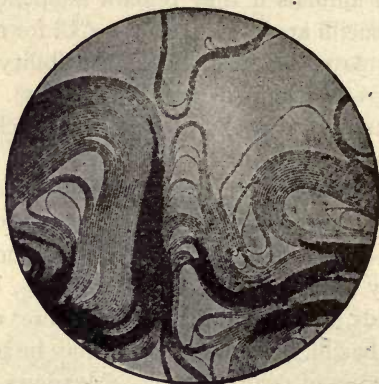


FIG. 53.—Anthrax bacilli. Cover-glass has been pressed on a colony and then fixed and stained. (Kolle and Wassermann.)

forms homogeneous flocculi, which precipitate, leaving the bouillon clear. A fragile pellicle is formed. In **milk**, it multiplies rapidly, the proteids are coagulated, generally rendered acid, and later the coagulum is dissolved. **Potato** cultures are likewise luxuriant. The growth is elevated, dull in lustre, and the outline is wavy.

Pathogenesis.—The anthrax bacillus increases so rapidly, and so luxuriantly, that it has been supposed to cause death merely by mechanically overwhelming the animal: absorbing nutriment and oxygen, and blocking capillaries. Its action is certainly not

purely toxic, as it causes, not a toxæmia, but a bacteriæmia. It is especially virulent for man, sheep, cattle, goats, rabbits, guinea pigs, mules, and horses. Rats rarely succumb. Pigeons, chickens, and dogs are immune. If frogs are kept at a temperature of 30°C. they become susceptible to infection. At their normal temperature they are immune. The disease produced by this organism is known variously in different countries as Anthrax, Splenic fever, Woolsorter's disease, Malignant pustule, and Charbon. It frequently devastates vast herds of sheep, cattle, and goats, and is often a pestilence in European countries, China, and South America. It appears sporadically in the United States. Its origin in this country can usually be traced to infection from hides or hair imported from abroad. The disease has been contracted from using shaving brushes made of insufficiently sterilized bristles. In man it is frequently fatal. The infection is first manifest as a small carbuncle or pustule, from this, rapid general infection, as a rule, ensues. In man and animals anthrax bacilli may be transmitted from mother to foetus via the placenta. The organism is found in enormous numbers in infected bodies, investing all the organs and the blood. Pus is produced by necrosis of tissue. Infection is accompanied by a high leucocytosis and fever. There is often congestion of the lungs; also an intense friability of the splenic pulp, and all the glands of the body become enlarged, and, at times, many of them suppurate. In woolsorter's disease, the bacilli are inhaled, and lung lesions result.

Immunity.—It is possible to immunize animals against infection with anthrax by means of vaccines. By this means the lives of many thousands of domestic animals have been saved. The vaccines are made by growing the bacillus at 42°C. for various lengths of time to attenuate them. An anti-serum has been produced by repeated injection of toxins and of sporeless rods. It is used locally around a pustule, and in doses of 50–100 c.c. intravenously. It is anti-toxic and seems to stimulate phagocytosis.

TETANUS BACILLUS

Bacillus Tetani.

Tetanus Bacillus (Fig. 54).

Lockjaw Bacillus.

First seen by Nicolaier, and isolated in pure culture by Kitasato.

Morphology and Stains.—Rod-shaped. Varying from 1.2μ in length, to very long threads of 20 to 40μ . Sometimes grow in

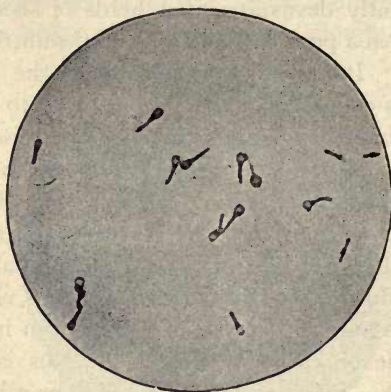


FIG. 54.—Tetanus bacilli showing end spores. (Kolle and Wassermann.)

chains; frequently appear like short drum-sticks with a spore at one end, which is either round or oval. At times, the bacilli in chains sporulate. The organism is motile; possesses numerous flagella (from 50 to 100) peritrichously arranged; stains well with all the common basic aniline dyes, and retains the color in Gram's method (Fig. 55).

Oxygen Requirements.—Strictly anaerobic when freshly isolated from earth or wounds, but, after long cultivation on culture media, it becomes more tolerant to small amounts of oxygen.

Temperature.—Grows best at 37°C . Below 14°C . not at all.

Vital Resistance.—Spores resist 80°C . for an hour. This fact

enabled Kitasato to kill all other organisms, except their spores, in pus. Six days' exposure to direct sunlight is needed to kill the spores. The thermal death-point is best considered as 100°C . for one hour. They are killed in two hours by 5 percent phenol + .5 percent HCl and in thirty minutes by 1-1000 HgCl_2 + .5 percent HCl.

Chemical Activities.—Ferments sugar; produces gas, indol, alkali, and H_2S . which gives to the culture an odor of burnt garlic or onion; marsh gas, CO_2 , and nitrogen are produced. Gelatine is liquefied. The most important product of growth is the highly poisonous complex toxin, which is made up of tetanolysin, and tetanospasmin; the latter has a great affinity for nerve tissues. This toxin is soluble in water, and can be separated from it by means of ammonia sulphate.

Habitat.—Is found in garden soil, hay, manure, and dust. Has been found in cobwebs, on weapons, in cartridges, and in the fæces of man and of animals. It has been isolated from bronchi in a case of rheumatic tetanus in which there was no lesion in the body (Carbon and Perrors). In **disease** it is found in the infected wound, generally in a deeply punctured one, which is usually purulent and contains but few bacilli. Puerperal tetanus, and tetanus of the new-born, are but varieties of the disease, dependent upon the site of infection whether of the placenta or umbilical cord. Tetanus sometimes occurs spontaneously, without a sign of injury anywhere. Sheep and goats are susceptible to infection, so are guinea pigs and rabbits. Horses are peculiarly susceptible. Soil, or manure, getting into wounds, is often a cause of tetanus. Cowdung poultices, mud dressings, or cobweb applications to stop hæmorrhages, have also caused the disease. Tetanus following vaccination may be due to infected virus, the latter becoming infected from the fæces of the vaccine-producing cows but more commonly is due to dirt getting into vaccination wounds.

Cultures.—This organism is difficult to grow, and always requires an atmosphere of hydrogen.

On **gelatine plates**, the colonies appear first as minute white specks, which slowly liquefy the medium. As it grows, hair-like threads branch out into the medium, and the colony resembles the periphery of a chestnut burr; later, the white appearance changes to yellow. In **gelatine stab** the growth is, at first, whitish along the line of the needle, eventually the gelatine becomes liquid, and a bubble of gas, partly filled with whitish-cloudy liquid gelatine, appears. On **agar plates** the colonies are ragged, and are surrounded by delicate out-spreading filaments. In deep stab culture, down in the agar and remote from the top, a spreading tree-like form appears, with spike-like growths in the agar. **Blood serum** is sometimes liquefied. **Bouillon** is uniformly clouded, gas is generated if sugar is present, and toxin is produced. **Milk** is, generally, not coagulated.

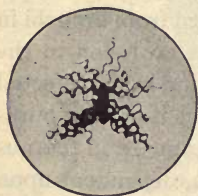


FIG. 55.—Tetanus bacilli showing peritrichous flagella. (Kolle and Wassermann.)

All cultures of tetanus must be grown under an atmosphere of hydrogen in media, from which all free oxygen has been driven by boiling, or else abstracted by a mixture of pyrogallie acid and sodium hydrate. It is possible to cultivate the organism under mica covering, or paraffine poured upon freshly boiled media. If sterile glass tubing is *filled* with agar or gelatine, and inoculated with tetanus bacilli, then sealed, colonies will develop, as perfect anaerobic conditions are thus obtained. Often the organism grows best in the presence of saprophytic ones. Strongly pathogenic organisms do not grow well in culture media, while comparatively non-virulent ones grow very well.

Pathogenesis.—Tetanus may follow any wound, no matter how insignificant, though deeply punctured ones, caused by nails or splinters, are more often followed by tetanus infection, especially if the puncture is sealed by blood clots or pus, and so creating an anaerobic condition necessary for growth. If the wound is on the

face or hand, tetanus symptoms more quickly supervene, while if the wound is on the foot, these are apt to be delayed. The sooner the symptoms appear after the reception of the injury, the more likely will the disease be virulent and fatal. If spores are washed free from toxin, according to Viallard and Rouget, and then injected into a susceptible animal, they do not cause tetanus, but are taken up by the phagocytes. In other words, the rods not the spores produce toxin. Necrotic tissue in wounds favors infection with tetanus, since it helps to fulfil anaerobic conditions, and in some way hinders phagocytosis. Aerobic bacteria favor tetanus infection by absorbing the free oxygen which prevents the growth of tetanus organisms. Free oxygen never kills the organism or its spores, but merely prevents their development. Wounds that have, apparently, healed, may be the origin of tetanus. The toxin is produced rapidly in wounds, or what is more likely, some is introduced with the bacilli and other dirt. Kitasato found, in the case of mice, that if bacilli were introduced in the skin, near the tail, and in an hour the whole area was excised, and the wound cauterized, fatal tetanus nevertheless supervened. Rheumatic tetanus follows pulmonary infection.

As related in the chapter on toxins, the mode of disease production is as follows: The toxin is conveyed from the wound by means of the motor nerves to the central nervous system affecting the motor elements. It causes microscopic degeneration of the fibers and cells of the motor apparatus. Death is caused either by a spasm of the glottis or diaphragm, or by cardiac failure and exhaustion. A local manifestation merely affecting certain groups of muscles may occur. Laking of the blood by tetanolysin found in the bodies dead from tetanus is a well-known phenomenon. In fatal cases, toxin may be demonstrated in the bladder by injecting the urine into mice, causing in them tetanic symptoms. Various groups of muscles are affected in tetanic seizures. The muscles of the jaw, if affected, cause *trismus*; if those of the back are involved the individual suffers

from *opisthotonos*. The seizures may be constant or *tonic*; or convulsive and violent, then they are designated as *clonic*.

Immunity.—Metchnikoff claims that the only natural immunity possessed by man against tetanus resides in his leucocytic powers of defense. Susceptibility of the natural receptors of the nerve cells for the toxin, and the degree of affinity, constitutes the cause of intoxication, its degree, and ultimate result. Affinity for the receptors of other less vital organs, on the part of the toxin, establishes a means of natural defense. Acquired immunity is dependent upon the formation of anti-toxin. The anti-toxin, formed by susceptible animals injected with tetanus toxin, is chiefly useful and valuable as a prophylactic measure. An epidemic of puerperal tetanus in a lying-in hospital was checked by its use. Sprinkling dry powdered anti-toxic serum on wounds infected with tetanus bacilli, or toxin, prevented infection (Calmette and McFarland). The anti-toxin may be injected either into the substance of the brain in cases of well developed tetanus, or into the cerebrospinal fluid, in the hope of neutralizing the toxin not already in firm combination with the nervous elements. Large nerves near the infecting wound may be injected with anti-toxin in the hope of binding the toxin already in combination with the nerve cells (see page 74).

Female mice immunized against tetanus toxin, transmit a great amount of immunity to their offspring. The milk of an immunized mouse also causes a passive immunity in other young that are suckled by her.

BACILLUS OF MALIGNANT ŒDEMA

Bacillus Œdematis Maligni.

Vibrion septique.

Bacillus of Malignant Œdema.

Morphology and Stains.—Thickish rods, resembling tetanus and symptomatic anthrax bacilli, with a tendency to grow in long

threads. It is actively motile, and is possessed of numerous peritrichous flagella. Spores are found which may be either equatorially or polarly situated. This organism is readily stained by the ordinary methods, but not by Gram's.

Chemical Activities.—Milk is coagulated, digested, and the reaction is amphoteric. Abundant alkali is formed at times; albumin is decomposed, forming fatty acids, leucin, an oil, and an offensive odor. CO_2N . and marsh gas, are also formed.

Habitat.—It is found in soil, dust, manure and dirty water and is widely distributed.

Cultures.—This organism is a strict anaerobic, and grows well in most culture media, at incubator or room temperature. On **gelatine plates** colonies develop on the surface (under hydrogen) in tiny shining white bodies, which upon magnification are found to be filled with a grayish-white substance composed of melted gelatine, and long tangled filaments. The edges of the colonies are fringed. In **gelatine stab** cultures (made in liquid gelatine, which, after inoculation, is rapidly solidified in ice water) a globular area of liquefaction occurred. If sugar is added, active fermentation takes place, with the production of large amounts of offensive gas. It grows well on **agar**, in **bouillon**, and in **milk**.

Pathogenesis.—Is pathogenic for man, horses, sheep, dogs, rabbits, calves, pigs, goats, rats, mice, and guinea pigs. Cattle are said to be immune. When bacilli are applied to a scratched surface, infection is not likely to occur, as free oxygen seems to inhibit the growth; if, however, the wound is deep, rapid infection follows, young domestic, and laboratory animals dying within forty-eight hours. The bacillus produces a moderate quantity of toxin and has an antagonistic action on leucocytes. In man, the clinical manifestation of infection with this organism is known as malignant Œdema. Infection has followed penetrating wounds of the body, by dirty tools, nails, splinters, bullets, etc. The disease is often quickly fatal. It produces, frequently, rapid moist gangrene.

The organisms and spores are not so very resistant as any strong germicide or a temperature of 100° acting a few minutes will kill them.

SYMPTOMATIC ANTHRAX BACILLUS

Bacillus Chauvoei.

Bacillus of Symptomatic Anthrax.

Rauschbrand Bacillus (Figs. 56 and 57).

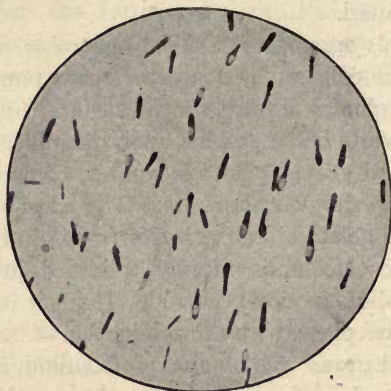


FIG. 56.—Rauschbrand bacilli showing spores. (Kolle and Wassermann.)

The cause of symptomatic anthrax, black-leg, or quarter-evil, in cattle.

Morphology and Stains.—This is a large organism, $.5\mu$ in width, and 3 to 5μ in length. It has rounded ends, and grows in pairs, but not in strings or chains. It is motile, and has many peritrichous flagella. When stained for spores, these bodies may be found distending the organism in the middle or at the end, and the bacillus assumes a drum-stick, or spindle shape. Often chromophilic granules are present; involution forms also appear, and are of enormous size. This organism stains with all the common

stains, but not by Gram's method. They may be seen in an unstained condition in blood or other fluids.

Habitat.—This bacillus is found not only in the diseased tissues and dead bodies of infected animals, but also in infected pastures, soil, hay, etc.

Temperature Requirements.—It is best cultivated at body temperature, but grows anywhere between 18°C . and 37°C . The spores resist boiling for half an hour but the vegetative rod is killed by 100°C .



FIG. 57.—Rauschbrand bacillus showing flagella. (Kolle and Wassermann.)

Cultures.—It is, like tetanus and malignant oedema organisms, a strict anaerobe. On **gelatine** it grows in roundish whitish colonies in a delicate tangled mass, with projecting filaments. The gelatine is liquefied, and bubbles of gas are formed in stab cultures. A sour odor is emitted from cultures; 1 to 2 percent of sugar is required for successful cultivation; or 5 percent of glycerine will answer. On **agar** the growth is marked; gas is produced, and acidous odors evolved. In **bouillon** it grows rapidly. Large masses of the organism sink to the bottom, gas is formed, and the medium is clouded. **Milk** affords a good medium for the growth of the organism, but the casein is not coagulated.

Pathogenesis.—Young cattle, six months to four years old, sheep, goats, rats, mice, and more especially guinea pigs, are susceptible to it. Swine are immune, while dogs, cats, birds, and rabbits are not susceptible. Man is immune. It causes in animals peculiar groups of emphysematous crepitating pustules, followed by emaciation and death. These areas contain dark fluid, probably broken-down blood. In guinea pigs inoculation is followed by death within thirty-six hours. The site of inoculation is found to be œdematous, and contains bloody fluid. The bacilli are mostly found at the site of the inoculation, but later in the blood in every part of the body. The virulence of this organism in culture media is soon lost. The addition of lactic acid to the cultures increases their virulence.

Immunity.—It is possible to decrease the virulence of this organism, and to use the weakened bacteria as a vaccine against infection. To attenuate this bacillus, prolonged exposure to heat, or to heat and drying together is necessary. Inoculation with bacilli treated in this way is followed by a mild local reaction, which affords complete immunity against infection with virulent bacilli. It has been found by Kitt that the muscles of an infected animal, if subjected to a high temperature—85°C. to 90°C.—afforded complete protection to the animal inoculated with them. It is best to use a weaker vaccine muscle that has been heated to 100°C. for two hours, in order to protect against the active vaccine. Before heating, the meat is ground. When used as an injection, it is crushed and mixed in a mortar with sterile water. Guillod and Simon found that this means of preventative inoculation reduced the death rate in unprotected animals from 20 percent to 5 percent. If this bacillus, and the prodigiosus bacillus are injected into naturally immune animals, death will often result.

There is a soluble toxin, anti-toxin against which appears in immunized animals. The toxin may be used for prophylaxis. One attack confers immunity.

MEAT POISONING BACILLUS

Bacillus Botulinus. *Van Ermengen.*

Bacillus of Meat Poisoning, or Botulism (Fig. 58).

Morphology and Stains.—This bacillus resembles thick vigorous rods, $4-9\mu$ long, and $.9\mu$ thick, is motile, has polar spores, and from four to nine peritrichous flagella. It is strangely called a saprophyte, because it is incapable of growth in the body, yet its toxin is highly poisonous to man and other animals. It is stained by all the usual basic aniline dyes, and by Gram's method.

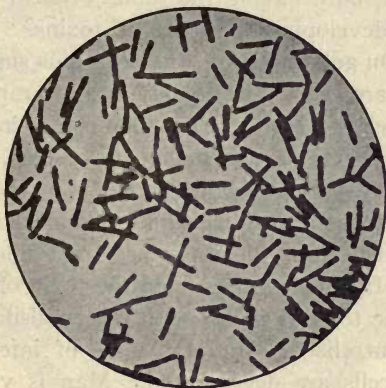


FIG. 58.—Bacillus of botulism. (Kolle and Wassermann.)

Habitat.—It seems probable that this organism occurs in the feces of animals, especially pigs, from which source it can gain access to the ground, to vegetables, or to the meat of the animal from which hams are cured or sausages made. While originally a disease described as originating from improperly cured hams, botulism has been known to follow the eating of tomatoes, beans and olives.

Vital Characteristics.—Is an anaerobe. Its thermal death-point, for a spore-bearing organism, is low, 80°C. , for an hour.

Grows only in media that are alkaline, and is capable of growth at from 18°C. to 35°C., though best below 25°C.; 10 percent of chloride of soda checks growth.

Chemical Activities.—It can produce, at room temperature, a water-soluble toxin sufficiently stable to withstand drying of meat if not exposed to sunlight, and not destroyed by the gastric juice. It is destroyed by thorough cooking of meat. Milk is not coagulated, grape sugar is fermented, and a foul, sour odor is produced in a culture. It liquefies gelatine. There are two varieties of the germ, A and B, differing in the quality of the toxin produced, both having the same physical and pathogenic properties but developing different anti-toxins.

Cultures.—On **gelatine plate**, that contains sugar, colonies are produced that are coarse and prickly in appearance. The liquefaction of the gelatine is slow. **Bouillon** is rendered turbid. The cultures resemble tetanus and malignant oedema.

Pathogenesis.—Its pathogenic action is marked, but only by its toxin, which has a decided affinity for nervous tissue. The toxin is absorbed from the intestinal tract unchanged by the gastric juice. In this it differs from the toxin of diphtheria and tetanus. If the toxin is mixed with the emulsified nerve tissue, it becomes neutralized. In fatal cases of infection, the ganglionic nerve cells are degenerated. Man is very susceptible, while cats and dogs are more or less non-susceptible. If bacilli are inoculated into animals, they do not proliferate. A meningitic disease of horses and limberneck of fowl are believed to be due to this intoxication. Animals that recover are found to have developed strong anti-toxin in the blood serum.

Immunity.—An artificially prepared anti-toxin has been found to be active, and is of use in treating cases of poisoning with meat. The correctly typed anti-toxin should be used or that made by artificial immunization with both varieties of bacilli. The latter is now preferred since no rapid distinguishing clinical test of types is available.

GASEOUS ŒDEMA BACILLUS

Bacillus Capsulatus Aerogenes.—Welch.

This description covers those organisms sometimes described under the title *Bac. perfringens* and *Bac. enteriditis sporogenes*; they are in all probably but variants from this type. Other types with slight variations in chemical action and toxin produc-

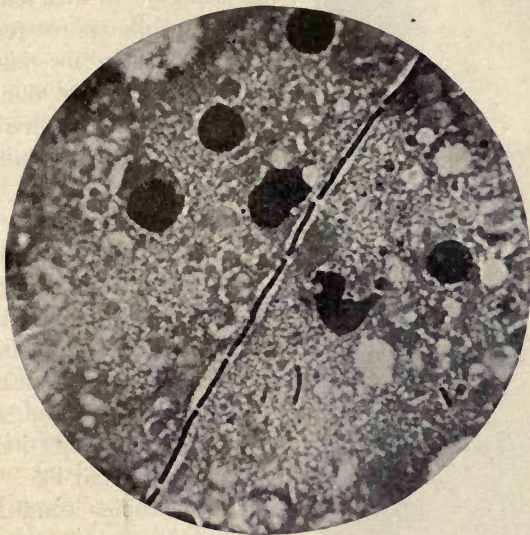


FIG. 59.—*B. Aerogenes capsulatus* of Welch, in smear. (Williams.)

tion were noted during the war. It is this group which was responsible for gas infection during the great European War.

Morphology and Stains.—A vigorous plump bacillus 3 to 4 μ in length, resembling the anthrax bacillus, and is usually straight. It forms spores, is non-motile, and flagella have not been found. It occurs in pairs, and in chains. In old cultures involution forms are seen. Spores are generally equatorially situated. Is colored

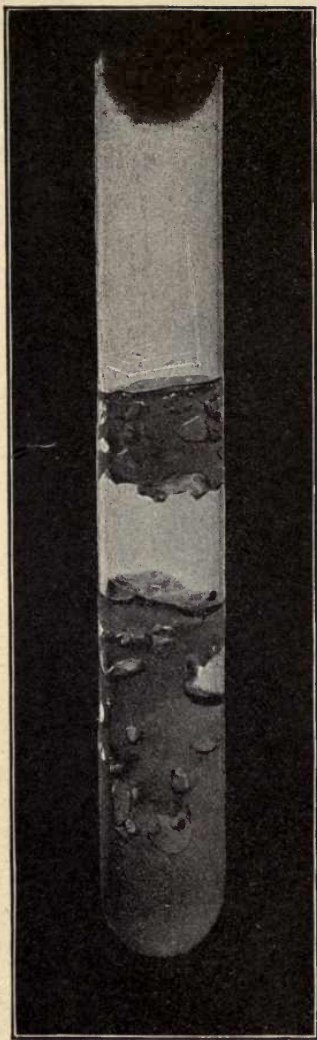


FIG. 60.—*B. aerogenes capsulatus*, agar culture showing gas formation. (Williams.)

by all the basic dyes, and holds the stain in Gram's method. Staining shows that it possesses a capsule.

Habitat.—The soil, the intestines, and, sometimes, the skin of man.

Vital Characteristics.—Vital resistance is low, the thermal death-point being 58°C . with ten minutes' exposure, while spores require ten minutes' boiling for killing. It grows best at body temperature. Has lived for one hundred days on culture media in the incubator. It is an anaerobe.

Chemical Activities.—Produces gas; does not usually liquefy gelatine, but curdles milk (Fig. 60).

Cultures.—Grows best in neutral or alkaline media, producing abundant gas. Colonies appear grayish or brownish-white, and are often surrounded by projections which are feathery or hair-like. On **agar** strict anaerobic conditions are necessary for growth, gas bubbles appear in the media, and the agar may be forced out of the tube in stab cultures. In **bouillon** it grows under anaerobic conditions. The growth is rapid, bouillon is clouded, and a froth appears on the surface. After a few days the medium becomes clear, owing to the sedimentation of the bacilli. Growth

occurs best in sugar bouillon, which becomes strongly acid. In **milk** the growth is rapid and luxuriant; the proteids are coagulated. Anaerobic conditions must be observed. On **potato** it grows well, producing bubbles in the water which may cover the potato in the tube. The growth appears thin, moist, and grayish-white.

Pathogenesis.—The pathogenic properties of this organism are limited. It is not able to endure the oxygen of the circulating blood. Grows best in old clots, and in the uterus. It produces gas rapidly in some cases of abortion and in peritonitis in man, which is quickly followed by death. It causes gaseous phlegmons in guinea pigs, and injection are usually fatal to birds. In man infection has followed wounds, and delivery of the child in puerperal cases. It produces in fatal cases the condition known as frothy organs—"Schaumorgane." It may be isolated from infected matter, fæces, etc., by injecting the latter into a rabbit's vein and then killing the animal. The carcass is then placed in an incubator and an enormous growth of the organisms follows; anaerobic conditions favorable to growth are obtained in the body so that gas distention of the tissues results; from the latter pure cultures are easily obtained.

Vincent's Angina is due to an anaerobic organism of two stages, as *Bacillus fusiformis* and *Spirochæta vincenti*. The bacillus is a fusiform irregularly staining pointed rod, 3-12 μ long by .3-.8 μ wide. Under cultivation it grows out into forms such as are seen with it in smears from the diseased throat, that is, long, wavy, uniformly stained, flexible, pointed ended spirals. The bacillus forms endospores chiefly at the end. Obligate anaerobe, requiring serum, ascitic fluid or glycerine. Colonies delicate and whitish. Gas in glucose media. Litmus milk only decolorized. Gives a foetid odor on all cultures. No specific immunity reactions known.

These same spirals have been found in abundance in many cases of ulcerative stomatitis, notably the variety which became

well known during the late war. Their connection with this so-called "Trench mouth" is not so generally accepted as is the case with ulcerative angina but their discovery should suggest remedies, salvarsan, silver nitrate, which have been useful in Vincent's Angina.

SPIRILLACEÆ

CHOLERA BACILLUS

Vibrio Cholerae. Koch.

Spirillum Cholerae (Fig. 61).

Cholera Bacillus.

Comma Bacillus.

Morphology and Stains.—Curved or bent rods, the ends not lying in the same plane. This bending varies greatly. Under

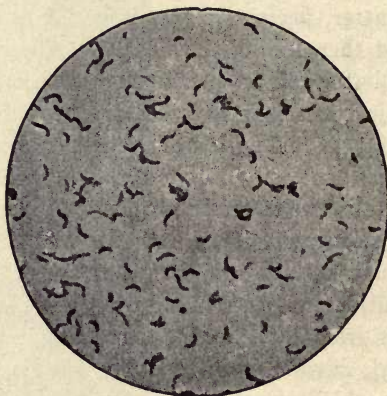


FIG. 61.—Cholera spirilla. (Kolle and Wassermann.)

certain conditions of growth such as the presence of alcohol, or insufficient albumin or oxygen in culture media, long spiral chains are formed. It is motile, has one terminal flagellum, and like other members of this family, has no spores. It stains well with the common dyes but not by Gram's method. Dilute fuchsin

stains it best. Occasionally involution forms are developed, which do not stain well. So-called arthrospores are formed, according to Hüppe.

Habitat.—It is said to exist constantly in the waters of the Ganges in India. Is found in contaminated drinking water, from rivers, lakes, and wells; also in human fæces, which, used as manure, infests vegetables, and spreads the disease. It is found in the intestines during cholera, and after death in other viscera. It can be disseminated by convalescent or healthy carriers; chronic carriers are not known.

Vital Resistance.—Is extremely sensitive to various deleterious agencies. Minute quantities of mineral acids, and other chemical disinfectants, as well as light, heat, and drying, quickly kill it; 1 percent carbolic kills rapidly. A 1-2,000,000 solution of corrosive sublimate destroys in from five to ten minutes. Its thermal death-point is 60°C. for ten minutes (moist heat).

Chemical Activities.—It creates indol in large quantities, and may be detected in peptone cultures merely by the addition of sulphuric acid. Lævorotatory lactic acid is produced from all the sugars. Gases are not formed. Yields alkali in culture; causes slight coloration of potato, and produces a disagreeable odor in bouillon; also yields H_2S , and ferments that liquefy gelatine. Bacteriolysins and invertin are also produced, as well as a toxin which is soluble in water. The most powerful toxin, by far, is contained in the cells of the vibrio themselves. This causes death after intraperitoneal injection in guinea pigs.

Oxygen Requirements.—It is a facultative aerobe; its growth, however, without oxygen is slow, while powerful toxins are formed.

Temperature.—Grows best at 37°C., but very well at 23°C. Does not grow below 8°C.

Cultures.—On gelatine plates the growth is characteristic. Small yellowish-white colonies, which rapidly liquefy the gelatine, appear in twenty-four hours. As the colony increases in size it

becomes more and more granular, and finally the whole medium is liquefied. In **gelatine tube** stab culture, the growth, at first, is not characteristic; but, after a few hours, a semi-spherical depression appears, which extends downward, and resembles a large bubble of gas. As liquefaction progresses, the whole line of puncture disappears, and the excavation looks cylindrical. This area becomes cloudy. On **agar plates** the colonies are elevated, round and white, with moist lustre. Deep colonies are whetstone shape. Old agar colonies become yellowish-brown. **Coagulated blood serum** is rapidly liquefied at 37°C. **Milk**, at times, is coagulated. No curdling ferment is formed; the acid produced is thought to be sufficient. On **potato** the growth is slow, or not at all, if the medium is acid. If the potato is rendered alkaline, growth occurs, with a moist lustre, slightly elevated; white at first, later becoming brown. On **acid fruits** it will not grow. In **bouillon**, after sixteen hours, a diffuse cloudiness occurs, with the formation of a stiff pellicle, which in some cultures becomes wrinkled. In **peptone**, abundant growth takes place, with the production of indol and nitrites. If a few drops of H_2SO_4 are added, a beautiful red appears if nitrites are present. This is the "cholera red" reaction. If the color does not at once appear, nitrites must be added. On **blood media** certain strains produce distinct hemolysis (El Tor).

Pathogenesis.—Cholera spirilla are pathogenic for man, but only under experimental conditions, for lower animals for which guinea pigs may be taken as an example. If the stomach of the latter is rendered alkaline with bicarbonate of soda, and a bouillon culture introduced, choleraic symptoms will follow and the animal will die. If cholera spirilla are injected into the peritoneum, the animal will quickly succumb to a general cholera peritonitis. Young rabbits are equally susceptible. When cholera spirilla in culture have been swallowed by man (laboratory workers), either by design or accident, the disease has followed, sometimes with fatal results. The toxin of this organism is intracellular (an endo-toxin). Old cultures become pathogenic through a bac-

teriolytic action, by which the cells are dissolved, and the toxin liberated. Filtrates from young cultures are non-toxic. If bouillon cultures are killed by chloroform, and then injected into animals, toxic action follows. In cholera the pathogenic process is mostly confined to the intestines. Toxic absorptions, due to the liberation of toxic products by the bacteriolytic action of serum, follow later. There is a desquamation of the epithelium of the bowel, and epithelial flakes found in the watery discharges resemble rice grains. Peyer's patches may become slightly swollen and reddened, and later, there may be diphtheritic necrosis above the ileocecal valve, and often a parenchymatous nephritis. The vibrios do not enter the blood.

Diagnosis.—Bacteriological diagnosis of cholera is accomplished by examining the alvine discharges. A mucous flake is mixed with some peptone solution, this is incubated, and the spirilla, if present, rapidly grow on the surface; after a few hours, plates are poured from this surface growth, and from the plates liquefying colonies are picked out, and bouillon cultures made. These are tested by serum, from horses artificially immunized by injecting cholera spirilla into them. If the organism under examination (after serum mixed with 2,000 to 3,000 parts of water is added) agglutinates, it is considered to be the cholera spirillum. Both in early and fatal cases, the agglutinating reaction is not available, since it takes some time for the agglutinins to form in the blood. Under the chapter on immunity an account of the Pfeiffer reaction is given, also one on vaccination against cholera infection, by means of killed cultures, under the chapter on vaccines.

Vibrios Allied to the Cholera Vibrio

Several other vibrios have been discovered that resemble the cholera vibrio. These are mostly found in potable water, and though in many respects identical with the cholera vibrio, they differ in essential points, *i.e.*, pathogenicity, and in their agglutinability with specific sera. The most important of these organisms

are: *Vibrio Metchnikovii*; *Vibrio proteus*; *Vibrio tyrogenum*; and *Vibrio schuylkilliensis*. There are no important pathogenic members of this group except the cholera vibrio.

GLANDERS BACILLUS

Bacterium Mallei.

Bacillus Mallei.

Glanders Bacillus.

Morphology and Stains.—Slender rods 2 to 3 μ . in length containing no true spores, but shining chromatophilic bodies (*Babes-Ernst* granules). In old culture, long club-like threads appear, which exhibit true branching. This organism is not motile, and has no flagella. It is stained with difficulty by ordinary methods, and not at all by Gram's method.

Vital Activities.—It is a facultative aerobe, growing feebly in the absence of air, and best at 37°C., in glycerine agar. Resists drying but feebly. Its thermal death-point is 55°C., ten minutes' exposure.

Chemical Activities.—Produces a brown pigment on potato, also mallein, and a little indol in old bouillon cultures. It forms no gas.

Cultures.—On **gelatine** it produces small punctiform colonies that are white, and become, after a time, surrounded by a distinct halo. The colonies are often very delicate and ragged. The gelatine is not liquefied. On **agar** the growth is best if glycerine is present, but is not characteristic. **Bouillon cultures** cause an abundant sediment, above which the medium is clear. **Milk is coagulated.** On **potato** the growth is characteristic. The color is, at first, yellowish-white like honey, becoming, finally, reddish-brown. The potato is much darkened.

Pathogenesis.—This organism is pathogenic for horses and man; 50 percent of men succumb after infection. Horses, asses, cats, dogs, sheep, and goats are susceptible in the order mentioned. Cattle and birds are immune. In horses the disease is known as

glanders, or farcy, and the avenue of infection determines the clinical form of the disease. The mucous membrane and the skin are the chief places of infection. A primary ulcer is formed in the mucous membrane of the nose, or in the skin. Subsequently, the lymph-glands and the lungs may be infected. Guinea pigs are easily infected. White and gray mice, and rats are immune. For purposes of diagnosis guinea pigs are inoculated, but care must be used, as several fatal cases have occurred in laboratory workers, it being a treacherous organism with which to work. In infected animals, it produces a rapid and marked inflammatory reaction, with the formation of pus. Certain "buds," or nodules are formed, which are between an abscess and a tubercle in structure.

The diagnosis of doubtful cases may be made by injecting the material into the peritoneum of male guinea pigs. A violent suppurative orchitis occurs from which the rods can be cultivated. The poisons are endo-toxic.

Agglutinations.—It has been shown by McFadyean that the blood of infected horses exhibits markedly agglutinative properties toward the glanders bacilli. Normal horse serum clumps often as high as 1-400. Diagnostic reactions should be 1-1000, supported by complement fixation and the Mallein test. A slight immunity is present after an attack. **Complement fixation** may be attained with the serum of an infected horse by using an antigen of glanders bacilli grown on glycerine broth, killed and filtered.

Mallein.—In old cultures a peculiar tuberculin-like substance (mallein) is formed from the bodies of the bacilli themselves, and in the bouillon. This is thermostabile and if injected into animals having glanders, produces a marked reaction (see page 86).

DIPHTHERIA BACILLUS

Corynebacterium Diphtheriæ (Löffler).

Bacillus Diphtheriæ.

Klebs-Löffler Bacillus.

Diphtheria Bacillus.

Morphology and Stains.—Long, bent, or curved bacilli of irregular contour, frequently clubbed or filiform at one or both ends; which contain chromatophilic granules, and often exhibit true branching; have no spores or flagella, and are not motile. According to Wesbrook, stained bacilli are of three types; (1) granular (containing the *Babes-Ernst* granules); (2) barred like a striped stocking; or (3) solid, staining uniformly throughout. The pleomorphic differences of various bacilli are most characteristic, and of diagnostic importance. This organism stains with all the basic

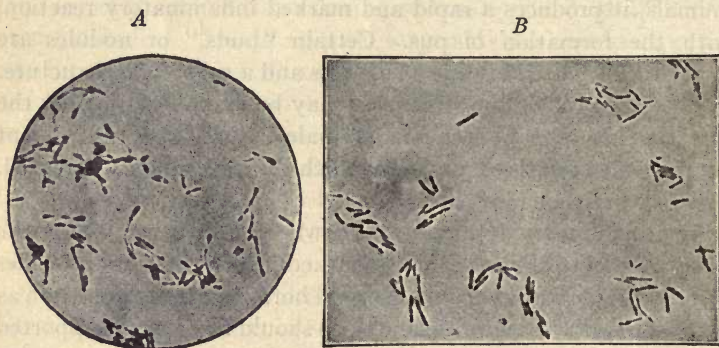


FIG. 62.—Forms of *B. diphtheriae* in cultures on Löffler's serum. *A*, Characteristic clubbed and irregular shapes with irregular staining of the cell contents. $\times 1100$. *B*, Irregular shapes with even staining. $\times 1000$. (After Park and Williams.)

dyes, notably by Löffler's blue, or Neisser's special granule stain. It is also stained by Gram's method. The length of the organism differs much, according to the reaction of the medium in which it grows. Alkaline media favor long forms, and acid the reverse. Its length is from 1.5μ to 3.5μ . It does not form chains. Bizarre, or involution shapes predominate in old cultures (Fig. 63).

Culture and Temperature Requirements.—It grows best at body temperature, and on glycerine agar, or in Löffler's blood serum mixture of alkaline reaction.

Vital Characteristics.—It resists drying for a long time, and has

lived on culture media for eighteen months at room temperature; also in silk threads for several months in a dried condition. Remains alive in healthy throats for months. Formalin vapor kills it speedily; corrosive sublimate solution, 1-10,000, destroys it in a few minutes; light is lethal to it in from two to ten hours, and heat at 58°C. in ten minutes.

Habitat.—It has not been found in sewage, or sewer gas, soil or water, the disease therefore is never transmitted by these means.

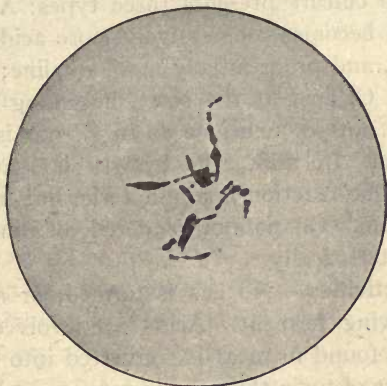


FIG. 63.—Diphtheria bacilli involution forms. (Kolle and Wassermann.)

Has been found in the throat, nose, and in the conjunctivæ of healthy bodies. In disease, the organism is mostly found in the throat, but has been isolated from all the organs in some fatal cases. Sometimes it is discovered in the throats of animals. Though its action is local, it elaborates a toxin which acts systemically.

Cultures.—On **gelatine plate** the growth is scanty and raised. This medium is never for cultivating this organism. The gelatine is not liquefied. On **glycerine agar plates** the growth, though moderate, is typically characteristic, but very slightly

raised above the medium, and is of duller lustre. Old colonies become yellowish-brown, the center of which, under a magnification of sixty diameters, appears darker, and with ravelled edges. On **Löffler's blood serum** mixture, the organism grows rapidly and well. This and **ascites-glycerine-agar** culture media are the best for it. **Bouillon** made from fresh meat is an excellent medium for its growth. The bouillon, which must be alkaline and freshly made, becomes first cloudy; then a fine precipitate settles, and over the surface a delicate pellicle forms. The reaction of the culture presents three types: A, is acid in the beginning, and becomes progressively more acid. B, is alkaline from the start, and progressively more alkaline; this is the most toxic growth. C, acid at the start, becoming alkaline finally. The growth is not so luxuriant as in B, nor is there as much toxin produced. In **milk**, the growth is luxuriant, without coagulation. The reaction is amphoteric, but in old cultures it becomes alkaline. On **potato**, rendered alkaline, it will grow, but not characteristically.

Chemical Activities.—No gas is formed, or any curdling or gelatine dissolving ferment. Acids are evolved from sugars; even the sugar found in meat is converted into lactic acid. In the manufacture of toxin this muscle sugar must be removed. A soluble toxalbumin is created, both in the body and in culture, which is intensely poisonous. See chapter on bacterial products.

Pathogenesis.—Diphtheria in man means generally an infection of the mucous membrane of the upper respiratory tract, with the formation of false membranes. The latter may cause death by suffocation. Infection may occur in the skin, vagina, or prepuce. The toxin not only causes a local necrosis, with the formation of an exudate, consisting of fibrin and leucocytes, but also grave systemic action, with marked degeneration of important nerves and nerve centers, and also of the parenchyma of the kidneys, liver, and heart, paralysis following. In certain structures fragmentation of the nuclei of the cells is noted. Guinea

pigs, cats, horses, and cows, may be infected artificially, but the disease never occurs spontaneously in these animals. Horses, dogs, and cattle are susceptible to its toxin. Diphtheria bacilli often have associated with them, streptococci, which add to their virulence, and complicate the disease. Endocarditis, adenitis, pneumonia, abscesses, and empyemia, may be caused by them. There may be puerperal diphtheria, due to the infection of the puerperal tract. Diphtheria is spread mostly by personal contact with individuals suffering from the disease, or with convalescents, in whose throats virulent bacilli linger, perhaps, for months. It may originate from infected milk, contaminated from human sources.

Perhaps the most important source of infection, especially during an epidemic, is the healthy bacillus carrier who, wholly unaware of his condition, is carrying virulent germs in his throat. This further indicates that individual resistance or susceptibility plays an important part in infection. (See Schick Test.)

Immunity is natural, active, artificial, or passive. Active immunity, following infection, is seldom permanent for although the individual, if he recovers, may be considered immune for a time, some individuals are more susceptible, and suffer several attacks. In active immunity anti-toxin is found in the blood, and recovery, and subsequently, immunity are due to this fact. Anti-toxin may be discovered in the blood, by mixing it with toxin of known strength, and injecting it into guinea pigs. If these survive a large lethal dose of the toxin, it is safely presumed that anti-toxin was present in the serum abstracted.

Passive artificial immunity is induced by injecting anti-toxin in the bodies of persons exposed to diphtheria. It is most effective but is short-lived, lasting only a few weeks. **Serum therapy** (see anti-toxin in previous chapter). If there is one natural specific cure for any disease, it is diphtheritic anti-toxic serum, which is prepared by immunizing horses with toxin, and abstracting their blood. The earlier it is given, the better are the chances of recov-

ery. As a prophylactic, from 600 to 1,000 units should be used. As many as 100,000 units have been injected in a single patient. No case is too trivial, or too far advanced in which to use it. The serum is anti-toxic, and not bactericidal. Wassermann has prepared a serum that is bactericidal, and is designed to destroy the bacilli.

Pseudo-diphtheria bacilli, which morphologically and culturally resemble the true bacilli, have been described. They are not pathogenic, in the sense of producing exudative diphtheria, and are believed to be attenuated diphtheria bacilli by many observers. The diagnosis of diphtheria by culture is an important measure. It depends upon the rapid growth of the bacilli upon Löffler's blood serum. Of all the various organisms found in the throats of patients with diphtheria, the diphtheria bacilli outstrip them in rapidity of growth. After eight to twelve hours, the serum inoculated with the smear from the false membrane is covered with fine granular colonies of pure diphtheria bacilli. After twenty-four, or more hours, the other organisms present overgrow the diphtheria colonies. A sterile swab of cotton, or a stick, is rubbed over the false membrane, or throat, and then over the serum; the latter is incubated, and the culture examined after eight or twelve hours, by staining with Löffler's blue. If curved, clubbed, irregularly stained bacilli are found, especially if they contain dark polar granules, and are generally uneven in size and bizarre, it may be safely considered that they are diphtheria bacilli. Gram's stain may be needed to confirm the diagnosis occasionally, or it may be necessary to inoculate guinea pigs. This may be done by inoculating the whole throat culture and plating out from the local inflammation, or the organisms may be isolated directly by plating and then injected into animals. It is well to check up the test by immunizing one animal with 200 units of anti-toxin before the culture is given, using another guinea pig unprotected. This serves as a control upon toxin production. Virulent bacilli will kill a guinea pig of 250 grams

in three days if 1 c.c. of forty-eight has serum broth culture be given subcutaneously.

Certain of the pseudo-diphtheria bacilli, or "diphtheroids" as they are called, seem to have the power of increasing or continuing inflammation after it has been started by other germs. They are frequently found in sinusitis, prostatitis, bronchitis, etc. In a few reported cases they seem to have been the only microörganismal cause of disease but their pathogenic powers are usually not great.

PSEUDO-DIPHTHERIA BACILLUS

Corynebacterium Pseudo-diphtheriticum.

Pseudo-diphtheria Bacillus (Hoffmann).

Morphology and Stains.—This bacillus resembles the diphtheria bacillus. The rods, however, are shorter and thicker; otherwise, it stains like the true bacillus, but not by Neisser's method.

Culture.—On glycerine agar the growth becomes diffuse, spreading from the line of inoculation in a grayish-yellowish pasty expanse. It grows well on gelatine. In bouillon it forms a denser and more luxuriant growth than the bacillus.

Habitat.—It is found in healthy throats and conjunctivæ.

Pathogenesis.—It is non-pathogenic for guinea pigs (see above).

Diagnosis.—It can be differentiated from the true bacillus by:

1. Being non-pathogenic.
2. Not exhibiting polar granules with Neisser's stain.
3. Not producing acids in certain carbohydrate media.

Bacillus xerosis is a pseudo-diphtheria organism found on the normal conjunctiva. It is not thought to possess any virulence.

TUBERCLE BACILLUS

Mycobacterium Tuberculosis.

Bacillus tuberculosis (Fig. 64).

Tubercle bacillus.

Morphology and Stains.—Slender rods, generally unbranched, 1–5 μ long, and 4 μ thick, usually slightly bent; are non-motile, and have no spores or flagella. In old cultures, and sometimes in sputum, branching forms are seen, and, rarely, some that are club-shape. On acid potato, thread forms are found. In the continuity of most of the bacilli, unstained spaces are seen; in



FIG. 64.—Tubercle bacilli in sputum; stained with fuchsin and methylene blue. (Greene's Medical Diagnosis.)

others dense deep red granules are found by fuchsin. As this bacillus is difficult to stain, special methods have been devised to demonstrate it, as the sheathing capsule renders it extremely unsusceptible to the ordinary methods of staining. The cause of this resistance is supposed to be a fatty or waxy substance in the capsule which is more than probable, because of the fact that stains that are fat selective, such as Sudan III, color it very well. Boiling hot carbol-fuchsin gives it the best stain. It keeps the color

in spite of the action of strong solutions of mineral acids in water, or dilute alcohol. So when tissues, or secretions, are stained with hot carbol-fuchsin for a short time, or cold carbol-fuchsin for a long time, and then treated with a 25 percent solution of HNO_3 , or H_2SO_4 , in water, everything is deprived of the red color, except the tubercle bacilli. All such organisms that are acid proof, are called "acid-fast." There are many other bacilli that have this property. Aniline water and gentian violet solution also stain it. Gram's method dyes the organism violet. Sometimes very young bacilli do not stain at all.

Vital Requirements.—This bacillus thrives best at 37.5°C. It grows slowly, is a strict parasite, and an obligate aerobe. In cultures it dies quickly in sunlight, and in diffuse daylight it dies in a few days. It resists drying and light in sputum for months. Its thermal death-point (moist) is 80°C. for ten minutes; can resist 60°C. for one hour, but succumbs to 05°C. in one

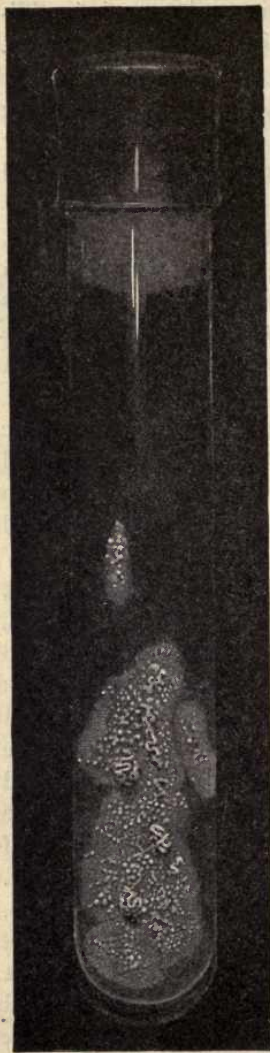
minute. It is quickly killed by formaline and corrosive sublimate, but resists 3 percent solution of carbolic acid for hours. In sputum it withstands antiseptics for a long time.

Chemical Activities.—It grows slowly, producing no coloring matter; yields an aromatic sweetish odor, but no gas or acid. It produces certain plasmins or endo-toxins, which are called tuberculins (*q.v.*).

Chemically the tubercle bacillus contains two fatty matters, one combined with an alcohol to form a 'wax. It has also a protamin, a nucleic acid or an albumose. Various fatty acids are to be derived from it by chemical treatment. The active principle in tuberculin centers around its protein elements, but is not exactly known.

Habitat.—It is a strict parasite and never leads a saprophytic existence. Is found wherever human beings live in crowded quarters; in dust of rooms, vehicles, and streets; and often in milk and butter. It is very widely distributed, being found in all human communities.

Cultures.—Since the organism does not grow below 30°C., gelatine is never used. On coagulated **blood serum** of cows, horses, and dogs, this bacillus grows best. As it is very difficult to isolate in pure cultures, the following procedure should be followed: The suspected sputum, fluid, or tissue is injected into a guinea pig, and when, in two weeks or more, large swollen glands can be felt in the groin, the animal should be killed, and a gland removed under strict aseptic precautions. It is then divided, and the halves containing the bacilli are rubbed over the surface of **coagulated dog serum** and allowed to remain in contact with it. The serum should be coagulated in special tubes, with glass caps, having small perforations, which are stopped with asbestos fibre, or glass wool. The organism grows well in air, but too great access thereto dries and kills it. After the tubes are incubated for a week or two, little scales growing unto clumps appear, which are lobulated and friable. At first white, it later turns



darker. This medium is never liquefied by the culture. On **glycerine agar** made of veal broth containing 6 per cent of glycerine, the organism grows well after isolation from the tissues, often luxuriantly (Fig. 65). A wrinkled film covers the surface of the agar, from which it is removed with ease. On **bouillon**, made of veal and glycerinized, it develops rapidly, covering the medium with a dense white wrinkled pellicle, which, though thick, is friable. After a time it falls to the bottom of the flask. It grows well on glycerinized potato also, and milk agar. On egg-albumins mixed together, sterilized and coagulated, this bacillus also develops well.

Pathogenesis.—The discovery of the tubercle bacillus, its methods of cultivation and differential staining, may be ranked with the greatest of medical discoveries. This organism causes in man and cattle, chiefly, the disease called tuberculosis. It rarely attacks the carnivora, but has been found in such animals when confined. Swine are often infected; cats and dogs sometimes, but sheep, goats, and horses seldom. It is easy to inoculate guinea pigs or rabbits by injection or feeding.

FIG. 65.—*Bacillus tuberculosis*; glycerine agar-agar culture, several months old. (Curtis.)

The disease is widespread, but is much more common where human beings are huddled together in dark, badly ventilated rooms and shops. In tissues, the characteristic lesion is a tubercle. This is a globular mass, about the size of a very small shot, and grayish pearly white. Microscopically, in the centre of the tubercle, are found several large multinuclear cells, called giant cells, which often contain thirty or more nuclei, and a number of tubercle bacilli, the nuclei often being situated at one pole, while

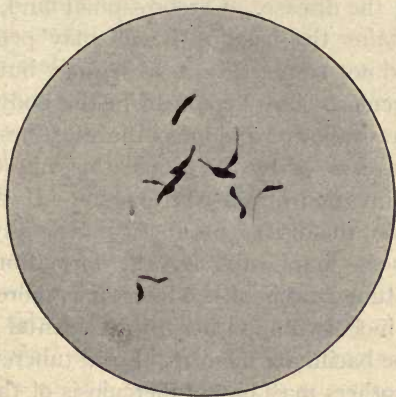


FIG. 66.—Tubercle bacilli showing involution forms. (Kolle and Wassermann.)

the bacilli are at the other. About the giant cells epithelioid cells are grouped, and about these small round cells are massed in great numbers. No new blood-vessel formation is ever found in the epithelial cell layers, or among the giant cells. Owing to insufficient blood-supply the centre of the tubercle frequently undergoes caseous degeneration. If the lesion heals, the caseous centres become calcareous, and the periphery changes into connective tissue. If the tubercles coalesce, great masses of caseous tissue form. If the latter becomes infected with other

pathogenic bacteria (streptococci and pneumococci) rapid softening occurs, with cavity formation, etc. Tubercles may develop in any organ or tissue of the body. The lungs, intestines, peritoneum, glands, larynx, spleen, and bones become infected. The liver and pancreas seem to resist invasion more than other organs. Bacilli are rarely found in the blood in tuberculous diseases. They may, however, be found in the urine, in kidney or bladder tuberculosis. Milk from tuberculous cows, with infected udders, often contains bacilli, and is certainly a means of transmitting the disease. Cerebro-spinal fluid, in tuberculous meningitis contains the bacilli. Bacilli may penetrate mucous membranes, and not causes any local lesions, but infect distant organs. Tuberculosis may be spread in the body in four ways. Sputum may be swallowed and infect the intestines, by continuity, by the lymph-stream, or by the blood; this may cause intestinal ulceration and invasion of the peritoneum. If the bacilli reach the blood-stream, the disease produced is generally acute miliary in type. This is manifested by the formation of fine gray tubercles. In tuberculosis of the lungs it is more than probable followed skin inoculation, either by accidental or intentional trauma that the bacilli are inhaled. Local tuberculosis has often Tuberculous mothers may have tuberculosis of the genital tract, and fathers, having tuberculous testes, discharge bacilli in the semen. Placental transmission of the bacilli from mother to child occurs rarely.

Types of Tubercle Bacilli.—It has been considered probable by many observers that there are two types of bacilli, a human and a bovine type. Theobald Smith was the first to advance this theory. Koch has announced that the two types were totally different, and that the human was incapable of infecting cattle, and the bovine was not pathogenic for man. In view of the fact that cattle are frequently tuberculous, and the bacilli are often found in the milk, it is important to know if the bovine type can develop in man. Ravenel has shown that it is undoubtedly

pathogenic for human beings. Men have been infected on the hands, while performing autopsies on tuberculous cattle, and their skin lesions showed, histologically, unmistakable tubercles. Cattle have been infected by bacilli of the human type. The bovine type of bacillus differs from the human in the following ways:

1. It is much more pathogenic for guinea pigs and rabbits.
2. It produces more extensive lesions in cattle.
3. It is shorter than the human.
4. It produces more alkali in acid media.
5. It is more readily isolated from original lesions and does not demand animal juices in culture media so emphatically.

The subject of the infectiousness of bovine tuberculosis for man has lately been exhaustively studied by Park and Krumwiede. Their conclusions are that bovine tuberculosis is practically a negligible factor in adults. It very rarely causes pulmonary tuberculosis or phthisis, which disease causes the vast majority of deaths from the spread of virus from man to man. In children, however, the bovine type of tubercle bacillus causes a marked percentage of cases of cervical adenitis leading to operation, temporary disablement, discomfort and disfigurement. It causes a large percentage of the rarer types of alimentary tuberculosis requiring operative interference or causing the death of the child directly or as a contributing cause in other diseases. In young children it becomes a menace to life and causes from $6\frac{1}{3}$ to 10 percent of the total fatalities from this disease.

It is not always easy to differentiate the tubercle bacillus from other pathogenic and comparatively harmless acid-fast bacilli. Among these are the *B. lepra*, the *B. smegmatis*, and a number of organisms found in butter, milk, hay, grass, and in the blind worm. Culturally, the difference is great. The surest way to differentiate the tubercle bacillus from other acid-fast organisms is by animal inoculations.

For the discovery of tubercle bacilli in materials apt to contain

other acid-fast several methods are now employed. The material to be examined may be stained in the ordinary manner and then decolorized by Pappenheim solution or a saturated solution of methylene blue in absolute alcohol. Preparations should be dried thoroughly before using such solutions. For "enriching" in organisms, the bulk of material, *e.g.*, sputum, is suspended in 15 percent antiformin (the proprietary name for a mixture of Javelle water and caustic soda), allowed to stand in the incubator for a while and the suspension centrifuged. In the sediment many more bacilli will be found than in the same bulk of the raw specimen. This antiformin seems to dissolve mucus, tissue and all bacteria except tubercle bacilli. The method can be used to procure cultures.

Even with this method organisms escape detection in some certainly tuberculous lesions. This is said to be due to non-acid-fast, but Gram-staining granules. They are said to be found by a modified Gram-Weigert staining, according to Much. Such specimens should always be injected into guinea pigs for corroboration.

Immunity.—It is possible to immunize cattle against virulent bovine tubercle bacilli by inoculating them previously with a culture of human tubercle bacilli that have been grown for some time on culture media, and thus attenuated. The tuberculins, if injected into a person with chronic tuberculosis, stimulate the tissues to a slightly greater resistance to the disease. Thus far anti-tuberculous sera are not of a pronounced or certain therapeutic value. By immunizing horses, Maragliano obtained a serum that he claims is effective. The milk from immunized cattle is used as a diet in tuberculous patients by him. The various tuberculins, some containing endo-toxins, or plasmins, in solution, are capable of stimulating the formation of agglutinins in the sera of man and animals. Blood from infected individuals also contains these bodies. The *agglutination test* does not seem to be of great practical diagnostic value, while the complement

fixation gives some information and is growing in favor as assistance in obscure clinical cases.

BACILLUS OF LEPROSY

Mycobacterium Lepra. Hansen.

Lepra Bacillus.

The original description is of a pointed, curved, acid fast rod occurring in groups within lepra cells. In recent years several different organisms have been isolated on media containing tryptophan. They have a few features in common: frankly acid fast or decolorized with difficulty. Gram positive, non-motile, non-spore forming, staining shows beading or barring, polar bodies, all capable of pleomorphism. They have been grouped into 4, (1) acid fast bacilli varying from coccoid to filamentous shapes, not easily isolated but growing well after once accustomed to media; growth yellow or orange; (2) acid fast, non-chromogenic, plump bipolar rods, short and long, growing with great sparsity on laboratory media; (3) diphtheroid bacilli staining solidly, or beaded, growing best at 37°C. in a yellow-white manner on agar and with a pellicle on broth; (4) anaerobic bacilli of more solid staining character and growing feebly as a dry band on media. Very marked variations in luxuriance and color production are noted on different media.

To cultivate the leprosy organisms bits of tissue are stripped off and allowed to digest with trypsin on blood serum or agar plates. When the tissue has softened and the bacilli multiplied, transfers are made to serum glycerine media or those containing tryptophan. It is best alkaline in reaction.

Pathogenesis.—The bacilli are seen in enormous numbers in lepra cells and elsewhere in diseased tissues and have been found in the blood. The lepra cells are large and vacuolated, and literally crammed full to bursting with bacilli. In general the leprosy

NOTE.—Tubercle bacilli causing avian and fish tuberculosis, and other acid fast bacilli exist, but not being pathogenic for man, are not described here.

lesion resembles a tubercle, as it consists of giant cells, epithelial, and round cells.

Immunity.—There is very little accurate knowledge as to immunity against this organism; of late bacterins have been tried with some success it is claimed.

STREPTOTHRIX (Eppinger) OR NOCARDIA

The genus of truly branching mycelium-forming higher bacteria (see page 3), such as the actinomyces, belonging to the group between bacteria proper and the moulds, called Trichomycetes. Kruse has described nineteen different members of the streptothrix, some pathogenic to man and animals.

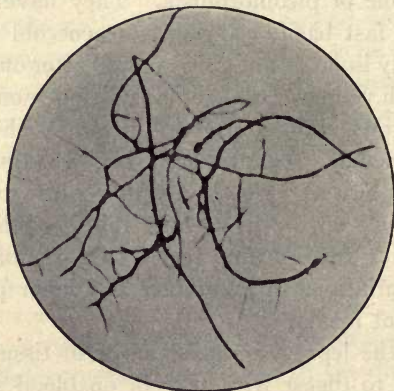


FIG. 67.—*Streptothrix candida*. (Kolle and Wassermann.)

A number of cases of streptothrix (*Streptothrix Hominis*) infection in man have been reported. The disease, in general, resembles phthisis. In the pus, sputum, and stained sections of these cases, streptothricial threads have been found (Figs. 67 and 70).

Morphology and Stains.—Threads are thick and short, or long and slender, depending upon the medium on which they grow. In **bouillon** the threads are thin and long, on **blood serum**, short and

thick. When stained there is distinct beading and fragmentation of the protoplasm.

There is true branching of an irregular type, which is best seen in liquid media. These threads often produce spores on culture media. The threads often disappear in old cultures, leaving only the spores, which stain with carbol-fuchsin and do not decolorize. The threads stain by Gram's method, and Gram-Weigert method. The threads are not acid-fast.

Vital Characteristics.—These organisms live for years in culture media after it is dry. Spores resist dry heat at 60°C. to 70°C. for an hour; moist heat, 60°C. however, kills them after an hour. It is a strict aerobe.

Cultures.—On Löffler's blood serum, according to Tuttle, this organism grows slowly in whitish colonies, which finally become yellow. The adult colonies adhere to the serum. On agar it grows rapidly and characteristically. The colonies are yellowish-white and adhere to the agar. **In Bouillon.**—It develops slowly on the surface of the medium. Fluffy tufts, or balls, are formed, that sink to the bottom of the tube. The growth is whitish.

Pathogenesis.—For rabbits and guinea pigs this organism is pathogenic, producing abscesses, tubercles, induration, etc. It is a pus forming organism.

In animals the spontaneous disease appears, best known as "farcin du boeuf," as ulcerative or infiltrative lesions of lungs and skin.

In man, the disease picture is like that of tuberculosis. It causes abscesses, adenitis, indurations of the skin, endocarditis, and pleuritic inflammation. Many grayish tubercles were found that resembled the lesions produced by the tubercle bacillus. Cavity formation has been described.

This organism may act as a secondary infecting agent in tuberculosis of the lungs. Tuttle reviews twelve cases, all of which were fatal.

In examining sputum from tuberculous cases, in which the typ-

ical bacilli are not found, it is well to look for the streptothrix by staining with Gram's stain.

RAY FUNGUS

Actinomyces Bovis.

Ray Fungus.

Morphology and Stains.—This organism is called the ray fungus because of the stellate arrangement of its threads in the colonies

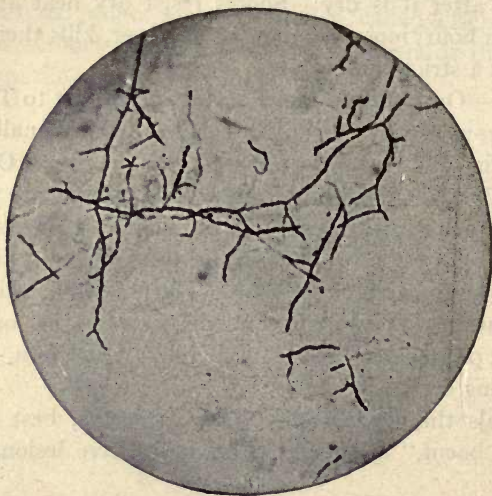


FIG. 68.—*Actinomyces bovis*. (Williams.)

found in tissues. It is of a more complex structure than the bacteria hitherto described. There are three elements found in every colony: (1) long thread which may be branched or unbranched; (2) threads that are clubbed, which may, or may not, be branched; (3) spore-like bodies contained within the thread, which seem to arise by breaking up of the threads. The colonies in tissues are often 1 mm. in diameter, and made up of many clubbed-shaped

threads radially situated. Through the periphery and extending beyond are other unclubbed threads, while scattered throughout the colony and beyond it, and in the threads, may be seen many spore-like bodies. The threads and spores stain by Gram's method while the clubs do not. Basic stains also color all the elements. The spores do not stain like bacterial endo-spores.

Vital Requirements.—It is a facultative anaerobe, and grows best in the absence of air, at 37°C. Resists drying for a long time, and its thermal death-point is 80°C. after fifteen minutes' exposure.

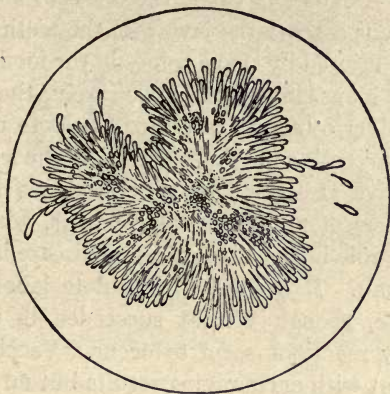


FIG. 69.—Actinomyces. (Williams.)

Chemical Activities.—Slowly liquefies gelatine, does not curdle milk; and produces a mouldy odor. No gas or acids are formed, nor is H_2S developed.

Habitat.—It has been found in straw and hay, but never in a healthy body.

Cultures.—On gelatine plates it produces yellowish-gray colonies that are very small. These grow into the gelatine, slowly liquefying it. The colonies are very tough and fibrous. In agar tubes it grows very slowly, the first growth being like dewdrops; later these enlarge, turning yellow, and finally brown. The cul-

ture grows down into the agar, and the medium darkens. Old cultures are dark and crumbly looking, adhere firmly to the agar, and have a downy dust-like covering. On **blood serum** the colonies appear as dewdrops, which later become brownish, then, yellowish-orange, or brick-red. In **bouillon** the growth is at the bottom in ball-like masses that cohere firmly. Clubs do not form in this medium. The supernatant bouillon is clear, with no surface growth. In **milk** it produces no chemical change. On **potato** it grows in knot-like colonies.

Pathogenesis.—Causes in cattle the disease known as “lumpy jaw.” The fungus reaches the jaw from the teeth and gums, the latter first being injured by sharp spines in the food. In man, the internal organs, lungs, intestines, and, rarely, the brain become infected. The liver often is abscessed. In both cattle and man universal actinomycosis sometimes occurs. The lesions produced are rather massive at times; the nidus is often surrounded by enormous numbers of polynuclear leucocytes, which, no doubt, play a defensive rôle in the tissues. The disease is often fatal to cattle and to man. It is hard to inoculate laboratory animals with the disease, though Wright succeeded in so doing. No useful immunity reactions seem to occur. Vaccines have been used in treatment with encouraging results but no cures. Potassium iodide internally is always indicated.

ACTINOMYCES MADURA

Actinomyces Madura.

Streptothrix Maduræ, Vincent.

Morphology and Stains.—A non-motile, non-flagellated organism said to have spores. Its growth resembles that of *Actinomyces bovis*. It consists of long threads that are clubbed. These stain by all the basic aniline dyes and by Gram’s method. There are three recognized forms of this organism, white, black and red, which have been found in various cases but the interrelation of

which is not yet fully understood. The description given is generally applicable only slight variations being noted.

Vital Requirements.—It is a facultative aerobe. The thermal death-point for the spores is 85°C. for three minutes, and 75°C. for five minutes. Vegetative thread forms die at 60°C. Grows best at 37°C., and scantily at room temperature.



FIG. 70.—*Streptothrix hominis*. (Kolle and Wassermann.)

Cultures.—Generates upon all culture media. **In Bouillon.**—It appears in little clumps which cling to the glass, but eventually sink to the bottom in masses. **In Gelatine.**—It grows sparingly in clumps, slowly liquefying the medium. **Upon Agar.**—It forms shiny round colonies, that are first devoid of color. They resemble an umbilicated vaccine vesicle and adhere tightly to the agar. **In Milk.**—It grows without coagulating the medium. **On Potato.**—The culture is very slow, and without chromogenesis. Old colonies are powdery, due to spores.

Pathogenesis.—In man it produces madura foot, an affection characterized by induration, ulceration, and fistulæ formation with pus.

BLASTOMYCOSIS

OIDIOMYCOSIS

Oidium Albicans. *Thrush, Soor.*—This organism resembles both a yeast and a mould, because it exhibits characteristics that are common to both of these forms. It exhibits budding yeast cells and budding mycelia. The yeast cell is 6μ long and 1μ wide, but the cells vary very much in length and width.

It stains well in tissues and cultures by Gram's method, and by the ordinary basic stains. It may be cultivated on bouillon,

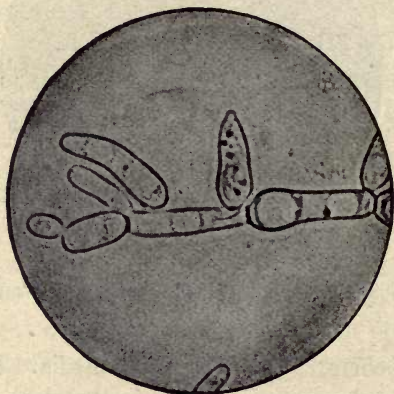


FIG. 71.—Thrush fungus. (Kolle and Wassermann.)

blood serum, agar, potato, etc., and it is rather indifferent to the reaction of the media. It grows best if sugars are present. It is, however, very susceptible to such antiseptics as phenol, salicylic acid, sublimate, etc.

Pathogenesis.—Causes in man a condition known as oidiomycosis, and in young children a very troublesome stomatitis, which, if the child is weak and illy nourished, may result seriously. It may cause metastatic abscesses in the brain, spleen, and kid-

neys, or nodules in the lungs. This organism may penetrate mucous tissues, and fill the lumen of vessels (Virchow).

Tropical spruce is held by some as due to a near relative of the oidium, namely *Monilia psilosis*. The organism is to be found all along the alimentary tract. It possesses many cultural characters like oidium. Vaccines are said to be of practical use in therapeutics.

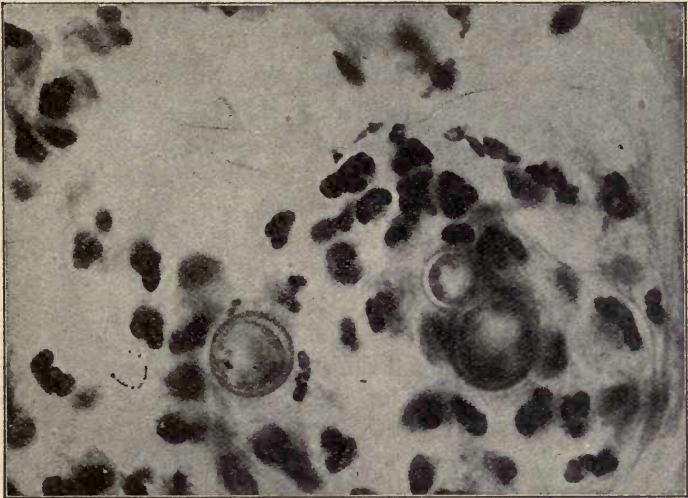


FIG. 72.—Doubly contoured organisms found in oidiomycosis (blastomycosis). (From Buschko after Hyde and Montgomery.)

Sporothricosis is a subacute or chronic infection usually of the skin, but at times involving the internal organs, caused by the *Sporothrix Schencki*. The organisms grow as delicate mycelia with very numerous spores, $2-4 \times 3-6\mu$ in size. They grow best upon acid media at body temperature as white fluffy masses which later become brown. The disease in man takes the form of firm tumefactions under the skin which may ulcerate leaving

indolent ulcers. Fever, prostration and emaciation follow. Rats are susceptible. An agglutinin appears; this may assist in diagnosis.

Saccharomycetes are sometimes pathogenic. They are budding fungi, multiplying by splitting off the bud when conditions are favorable for active growth but capable of intracellular sporulation, ascospores, when under adverse conditions. They usually have a rather resistant capsule, sometimes double. *Saccharomyces busse* or *hominis* is capable of setting up in man a cutaneous and subcutaneous ulcerative and infiltrative or even suppurative lesion which may last for a long time; involvement of internal organs can occur. Transmission to animals is difficult. The organisms are from 3 to 30μ , round or elliptical, rarely forming mycelia in the tissues. They stain well but not by Gram's method. They are best seen by mixing the pus with a caustic solution. They grow under aerobic conditions at 37°C . upon acid serum, dextrose or maltose agar as white plaques which later become wrinkled and velvety. They are easily killed.

Coccidiosis or oidiomycosis is an infection very similar to the foregoing but the causative organism, *Coccidioides immitis*, differs from *Sac. hominis* in showing intracellular sporulation and no budding.

MOULDS OR HYPHOMYCETES

These are the next higher order of plant algæ and consist of cells which can elongate to threads, dividing by intracellular sporulation or by the development of reproductive organs which in some varieties are bisexual in character. They are widely distributed in nature living mostly as saprophytes. Diseases due to these forms are practically confined to the skin although extremely rare cases of dissemination are on record.

Ringworm of all kinds is due to the mould *Trichophyton* either of the species *megalosporon* or *microsporon*. The spores of the former are $7-8\mu$, of the latter $2-3\mu$. They grow readily as dis-

crete mammillated fluffy colonies. They consist under the microscope of slender septate hyphæ.

Favus is due to the mould *Achorion Schoenleinii*. This fungus gives off hyphæ with knob-like reproductive organs. Spores are oval $3-8\mu \times 3-4\mu$. This fungus grows as a "scutulum" on the skin eruption. It can be cultivated on sugar agar, as a waxy, or downy yellow or white round plate with a central mammillation.

Pityriasis versicolor is due to the mould *Microsporon furfur*. It is similar to the Trichophyta, but invades only the superficial layers of the skin.

Aspergillus Niger, *A. Fumigatus*, and *A. Flavus*.—A polycellular mycelial organism which produces spores and branched threads, that are variously named from the macroscopic appearances of the growth. All thrive well at 37°C . and may be cultivated on the usual culture media. In man, the external auditory meatus is often infected with these organisms, causing a troublesome disease. They may infect the lungs of weak anæmic subjects with wasting diseases, and may be pathogenic for cattle, horses, and birds.

The author has found that the young hyphæ, the sporangia and spores of some of these hyphomycetes (moulds) if treated with hot or boiling alkaline solution of copper sulphate are stained by the copper, which has an affinity for them, and appear a light lilac-blue under the microscope. If treated with a solution of ferric cyanide of potash and acetic acid, these stained parts turn a dark brown, showing that there is an actual absorption or perhaps chemical union of the protoplasm of the mould with the copper. Some moulds are stained a deep blue, and are visible to the naked eye in test-tubes, after treatment with the boiling alkaline copper others are colored a bright yellow. Some moulds and bacteria have the power of reducing copper in Fehling's solution.

CHAPTER IX

ANIMAL PARASITES

While numerous diseases are caused by vegetable parasites, such as bacteria and moulds, there are others in which the etiological rôle is played by minute microscopic organisms of the animal kingdom. There are also infectious diseases that are supposedly caused by animal parasites, and yet the exact knowledge that they are the cause is lacking. Not all of the pathogens of the animal kingdom will fulfil Koch's postulates but their number is increasing. Within the past few years it has been found possible to cultivate Trypanosomata, spirochætæ, amœbæ, and hemosporidia with completion of Koch's postulates in the first two.

In general, it may be said of animal parasites, particularly those belonging to the protozoa, that an intermediate host, such as a suctorial insect, is necessary for the transmission of the organism to man or animal. This is called alternate generation and is a very characteristic feature.

The protozoa, as parasites in man, are the cause of several well-known diseases, namely: dysentery, malaria, sleeping-sickness, and coccidiosis. In hydrophobia, scarlet fever, and small-pox certain peculiar bodies are constantly found that resemble protozoa, but since it is not known whether they are animal bodies at all, they cannot be classed as protozoa, and are discussed under Chlamydozoa in the next chapter.

PROTOZOA

The protozoa of importance as disease producers are to be found in the classes, orders and families given as follows:

Protozoa.

Sarcodina.

Rhizopoda.

Amœbina—Amœbæ.

Mastigophora.

Flagellata.

Monadida, Cercomonas, Trypanosoma, Polymastigida, Trichomonas.

Some authors separate a family Spirochætidae to include Spirochæta and Treponema.

Sporozoa.

Gregarinida—gregarines.

Coccidia—coccidia.

Hemosporidia.

Plasmodium—malaria.

Infusoria.

Ciliata.

Heterotrichida—Balantidium.

The **protozoa** are always, in every stage of development, primitive unicellular bodies. They consist essentially of a cell body or *sarcode*, a *nucleus*, and a *nucleolus*. All of the vital functions of the cell are carried out by the cell body, the protoplasm of which digests and assimilates food. Particular parts of the protoplasm have special functions, these parts are called *organelles*. The living protoplasm is finely granular, is viscid, and exhibits a distinct movement. The motility of protozoa is supplied variously. In the Rhizopoda progression takes place by pseudopods or false feet, a phenomenon in which a section of the cell wall and protoplasm are extended like a bud. Into this the latter then flows with a shrinkage of the main body. At last the pseudopod is large enough to hold all the protoplasm and the former place of the protozoon is vacated for the new. Motility is also supplied by the lashing or vibratory action of flagella or the fine vibration of

circumferential cilia. In others a special muscular segment of the body may exist. The suckorial tubes act also for motion at times. In most protozoa two layers can be seen—the *ectosarc*, and *endosarc*. The *ectosarc* originates the movement, is concerned in the ingestion and excretion of food, and the respiration. The *endosarc*, which circulates slowly, is mainly for digestive purposes. In it are ferments, crystals, food particles (seen in the *food vacuoles*), oil globules, gas, and pigment granules.

Flagella and suckorial tubes—in protozoa that have them—belong to the *ectosarc*. Skeletal tissues, shells, etc., also belong to this layer.

The food consists of bacteria, smaller animals, algæ, and animal waste.

Propagation is effected by direct cell division, beginning in the nucleus, by cell budding or by a complicated course of sporulation which may be sexual or asexual. Sometimes division, or budding, occurs rapidly without the segments separating, leading to the formation of protozoal colonies, or swarm spores.

In the case of the malarial plasmodia, asexual development, (*schizogony*) takes place in man's blood, while the sexual development (*sporogony*) takes place in the mosquito. Protozoa are found in salt and fresh water, in damp places, and in animals as parasites.

Since the zoological classification has been given and may be used for reference to larger works, the various pathogenic protozoa are given separately without direct reference to their systematic classification.

There are but two Rhizopods that are parasitic and pathogenic to man. The only one of these of any import is the *Amœba*.

AMŒBA DYSENTERIÆ OR ENTAMŒBA HISTOLYTICA

This is a pear-shaped roundish body from .008 to .05 mm. in diameter. The *ectosarc* is easily discernible in the pseudopodia,

but not in the round quiescent cell. In the endosarc, which is granular, vacuoles are easily seen; so are fragments of food, red and white blood cells, bacteria, epithelial cells, and fæcal matter. The pseudopodia are broad and lobose; one or two are protruded at a time. The motion of the organism depends upon the reaction of the media, and the temperature. The vacuoles and nucleus are always present. Propagation generally takes place by binary division, the process beginning in the nucleus. When irritated, the amœba at once assumes a spherical form, the pseudopodia being withdrawn.

Pathogenesis.—Amœba dysenteriae is the cause of the protozoal form of dysentery. So far as known this particular variety exists only in the intestines of effected persons. Lesions similar to those of human dysentery have been produced in monkeys, dogs and cats, and the amœbæ recovered from them. Cultures consisting only of amœbæ have been obtained by special technique, but a so-called pure mixed growth of colon bacilli and amœbæ is cultivated with little difficulty. In the lower gut of man and cats, in dysentery cases, encysted amœbæ are often found. They have been seen in the liver (in old cases), also in the lungs and sputum.

Cats have been infected by pus from liver abscessed devoid of bacteria (Kartulis). The urine, in cases of cystitis, contained amœbæ, and it is believed to be the cause of the disease in some rare instances. In dysentery the amœbæ are the cause of the necrosis and ulceration, as they frequently become encysted in the submucous tissues. From the *Entamœba coli* the dysenteric amœbæ is differentiated by the fact that it is larger, coarser in structure, and takes up red blood cells, which the former does not. Differentiation by Wright's stain *Entamœba coli* ectoplasm light blue, endoplasm dark blue, nucleus red. *Ent. histolytica* ectoplasm dark blue, entoplasm light blue, nucleus pale red or pink.

Amœbæ quickly lose their mobility as the surrounding temperature leaves that of the normal body, although they are not

killed at low temperatures, and resist up to 60°C. when in the encysted state. Quinine, permanganate of potash, weak acid and silver nitrate are quickly fatal to vegetative forms. Emetin is a useful drug since it kills all but encysted forms after a very short exposure if direct.

Entamoeba tetragena, formerly classified as a separate variety, is now generally believed to be but one stage in the development of *Ent. histolytica*.

In stools (from dysenteric cases) over a day old, amœbæ are not often found, as they undergo a rapid disintegration outside the body.

Amœbæ are cultivated upon stiff agar preferably with defibrinated blood and in company with bacteria. If a colony can be obtained free of bacteria, development will continue on agar

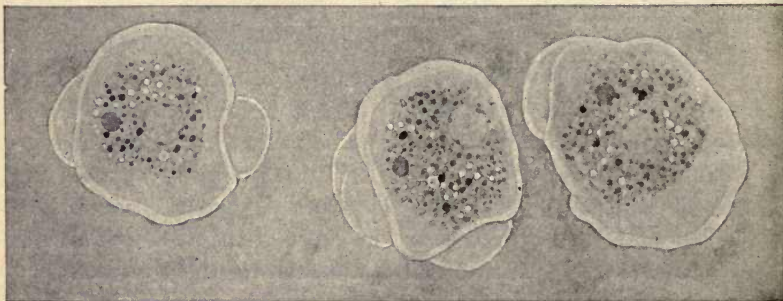


FIG. 73.—*Entamoeba tetragena*. The same living individual drawn at brief intervals while moving. (From Doflein after Hartmann.)

smeared with organ extracts. The addition of dead bacteria to culture media seems favorable to their development. The poison is not known. The free amœbæ in the colon are easily killed, but when encysted are more resistant. Quinine is fatal to cultures in ten minutes in strength of 1-2500. Formalin is not practicable.

Endamoeba buccalis is found in the mouth especially in carious

teeth and in inflamed gums; it may have some effect in continuing a gingivitis.

FLAGELLATA

The flagellata derive their name from the fact that all are possessed, at some time in their existence, of flagella, which are not only organs of locomotion, but serve to apprehend food.

The principal members of this class of interest from a pathological viewpoint, are the trypanosomes. *Trypanosoma gam-*

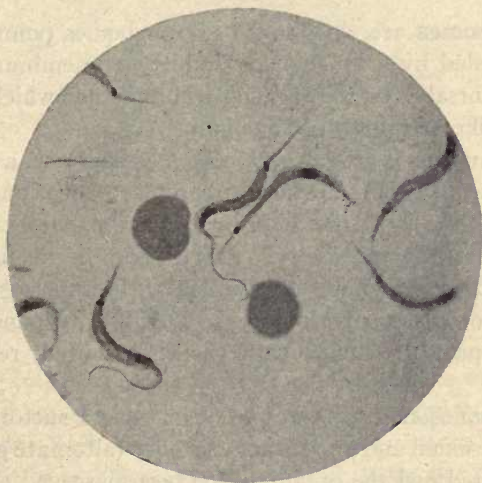


FIG. 74.—Trypanosome in rats' blood. (Williams.)

biense, transmitted by the tsetse-fly *Glossina palpalis*, pathogenic for man (see page 239). The *Trypanosoma brucei*, which causes the tsetse-fly disease (nagana) in horses and cattle, is transmitted to cattle by the bite of the tsetse-fly, *Glossina morsitans*. It can be grown on blood agar (Novy).

Trypanosoma evansi causes surra, a disease of horses in Central Asia transmitted by a fly of the genus *Stomoxys*.

Trypanosoma equiperdum causes a sexual disease in stallions and mares called dourine; this is akin to syphilis in man.

Trypanosoma lewisi of rats is transmitted from animal to animal by means of fleas.

Trypanosoma noctuæ.—A parasite of the little owl, which is introduced into the bird through the bite of the mosquito *Culex pipiens*.

Several other forms have been noted but these are perhaps the most important and serve as examples for this family of protozoa.

Trypanosomes are elongated fusiform bodies pointed at both ends, provided by a fin fold, or undulating membrane, running along the dorsal edge and forming frill-like folds which terminate in a whip-like extremity or flagellum.

A large nucleus is always seen, also a centrosome, a small chromatic mass—likewise called a blepharoplast—near one pole.

The flagellum is at the anterior extremity; the blunt pointed end is the posterior extremity. Cell division begins in the blepharoplast, the cell dividing longitudinally, the nucleus, flagellum, and the protoplasm dividing last. Dividing trypanosomes frequently appear in clumps with the ends united, resembling a wheel.

The trypanosomes exist in two hosts—one a suctorial insect—and have a sexual and an asexual existence (alternate generation). In an infected owl the organism has been observed clinging fast to the red cells, absorbing nutriment during the day, while at night it swims about freely in the plasma.

In owl's blood the trypanosome assumes asexual forms, called *macrogametes*. These macrogametes penetrate the erythrocytes, accumulating the remnants of the red cells in the protoplasm. The nucleus of the trypanosome may be seen in the interior of the protoplasm. The microgametocytes arise from the asexual forms and when mature, give rise to eight microgametes.

TRYPANOSOMA GAMBIENSE

Castellani found that this trypanosome is the cause of sleeping-sickness among the natives of South Africa, and the organism has been found quite regularly in the blood, and also the cerebro-spinal fluid sometimes as well, in this disease. The disease has a long period of incubation (months), runs a long course usually, and, at its full development, it is a meningo-encephalomyelitis. This is characterized by hebetude, somnolence, and coma.



FIG. 75.—Trypanosomes; showing ordinary structural appearance on left; in middle a trypanosome undergoing division; on the right a group dividing in radial manner. (Tyson's Practice.)

These symptoms are accompanied by disturbance of the motor apparatus, œdema, irregular temperature, rapid pulse, emaciation, skin eruptions, and death in coma. In these cases the parasites may be seen in the blood slowly winding their way through the corpuscles. The pathogenic action is due no doubt to some toxin elaborated.

The disease is transmitted from man to man by the tsetse-fly (*Glossina palpalis*). In the fly it exists as a true parasite in a host, and not merely passively. It becomes infective within three days of biting and remains so for four weeks.

The disease does not depend upon the age, sex of the individual, nor upon drinking water, food, seasons, etc.

The organism may be stained by the ordinary blood stains,

mixtures such as Leishman's, Romanowsky's, etc., the nucleus, centrosome and flagella, staining deepest. Thus far the *T. gambiense* has not been cultivated in artificial media.

Novy has succeeded in growing the *T. lewisii* and *T. brucei* on agar mixed with defibrinated rabbit's blood. These are the first animal parasites to be cultivated artificially.

Trypanosomiasis of South America is not unlike sleeping-sickness of Africa. It is caused by *Tr. cruzi*, a parasite of eight spores developing in organs, serum or red cells. It is transmitted by *Conorrhinus megistus*, a large insect.

In **Dum Dum** fever or **Kala Azar**, a disease characterized by wasting, anemia, fever and splenomegaly occurring in India, curious bodies, called Leishmann-Donovan bodies, have been found. These resemble the malarial plasmodia roughly, and if cultivated on blood agar elongated herpetomas-like bodies without undulating membranes will develop. They are to be found in the juice obtained by splenic puncture lying within cells, especially endothelium and large lymphocytes; on rare occasions they have been met in the blood. The transmission is not certainly known but may be by the bed-bug or by fleas.

Trichomonas vaginalis and *intestinalis* are flagellates which are apparently able to set up some inflammatory irritation in the places indicated by their special names.

TREPONEMA PALLIDUM (*Schaudinn*)

(*Spirochæta Pallida*.)

Treponema Pallidum.—There has been some discussion as to the proper classification but now this organism is usually placed among the Flagellata, genus *Treponema*. It does not possess an undulating membrane, is flagellated, is of stiff and regular shape, and multiplies by longitudinal division.

Morphology and Stains.—This organism is extremely delicate in structure, from 4 to 14 μ in length and about .3 μ in width; has

from 3 to 12 turns or bends, and its ends are delicately pointed. Its curves form a large arc of a small circle; the *Sp. refringens* curves form a small arc, frequently irregular, of a larger circle. It multiplies by both transverse and longitudinal division. As this organism is stained with difficulty it requires a special one, that of Giemsa yielding the best results. Aniline gentian violet, Romanowsky's, and Leishman's stains also color it. It may be stained in tissues by silver and pyrogallic acid methods.

Habitat.—It has not been found in tissues of normal persons, or those ill with carcinoma, tuberculosis, etc., but only in the tissues of individuals suffering with syphilis. It is a strict parasite.

Vitality.—The organism is readily destroyed by the ordinary disinfectants and dies after a few minutes' exposure to 50°C.

The *Treponema pallidum* has now fulfilled the postulates of Koch. It can be cultivated from human lesions (with some difficulty to be sure), it can be implanted in animals (monkeys and rabbits) and there reproduce syphilitic lesions; and it can be re-cultivated from them. In these experimental diseases it retains the proper morphology. According to Noguchi there are two types, a slender and a stout, which breed true to these characters and correspond to slight pathogenic variations. Noguchi succeeded in cultivating the Tr. pall. in pure culture by using the juice from human or monkey's lesions or from the syphilitic orchitis of rabbits. This he grows in serum water or serum agar to which has been added fresh tissue of rabbit. The organism grows as fine fibrils in arborescent colonies. These can be selected pure by cutting the tube and the agar column. Motion is of screw and serpentine character. No odor or spores are produced. This organism must be imagined and remembered as a corkscrew and not a waving line. The Gram stain is negative.

From the cultures of this organism a toxic extract can be obtained which, when rubbed into the skin of a syphilitic in the late stages, gives a typical skin reaction, luetin and the luetin reaction.

The *Spirochæta refringens*, which has been also cultivated by Noguchi and thought by him to be a *Treponema* also, grows without fresh animal tissue in a short time and produces no odor.

Pathogenesis.—It has been found in chancre, condylomata, and mucous patches in the early stages of syphilis; also in the blood, blister-fluids, spleen, bone marrow, liver, thymus gland, and

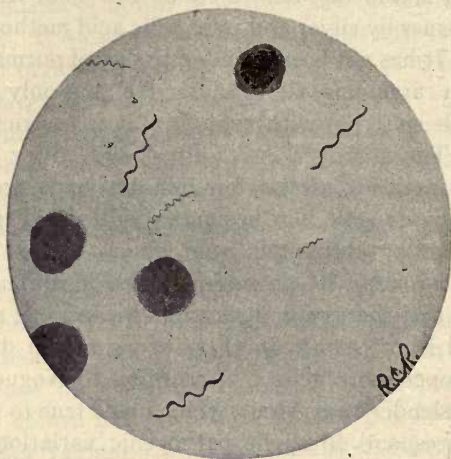


FIG. 76.—The *Spirochæta refringens* is the larger and more darkly stained organism, while the lightly stained and more delicate parasite is the *Spirochæta pallida* (*Treponema pallidum*). From a chancre stained with Wright's blood stain. (Hirsch—by Rosenberger.)

lymphatic glands, and in the brain and cord of taboparetics. Associated with this organism, in nearly every case, is a coarse-looking larger spirochæta (*Treponema*), which stains deeper, and has been called the *Spirochæta* (*Treponema*) *refringens* (*q.v.*).

In a series of experiments, Metchnikoff and Roux caused abortion of the chancre following inoculation of syphilitic virus on the eyelid of a chimpanzee, by calomel inunction carried out less than one hour after the infection; a solution of sublimate has not the same prophylactic property.

It does not require any intermediate host for transmission as do the recognized animal parasites of malaria and filariasis, etc.

Treponema pertenue is the organismal cause of Framboesia or Yaws, a cutaneous and general infection of the tropics similar to syphilis. It is about the size and shape of the syphilis spirochete but is distributed differently in the human lesions, being more in the outer skin and less in the vicinity of blood-vessels. It has not been cultivated.

Spirochæta nodosa (Huebner) or **icterohemorrhagica** (Inada) is believed to be the cause of Weil's disease. The spiral, a tiny organism about 5 micra long is found in the blood, liver and kidneys. It is transmissible to guinea pigs. Rats are supposed to be the means of transfer since several varieties probably harbor the parasite. Excretion of the organism takes place via the kidneys. Spirocheticidal substances occur in the blood in a favorably progressing attack.

Spirochæta morsus muris and **Sp. muris ratti** are supposed to be the cause of rat bite fever. These organisms, resembling but grosser than the *Treponema pallidum*, enter with a rat bite and can be found in swollen drainage lymph nodes.

RELAPSING FEVER ORGANISM

European Relapsing Fever.—Caused by **Treponema** of **Spirochæta obermeieri**.

African Relapsing Fever.—Caused by **Trep.** or **Sp. duttoni**, transmitted by tick **Ornithodoros moubata**.

American Relapsing Fever.—Caused by **Trep.** or **Sp. Novii**.

Bombay Relapsing Fever.—Caused by **Trep.** or **Sp. carteri**. The transmission of the first, third and fourth, while not definitely known, is probably by a louse; ticks may also be responsible.

Morphology.—They have lately been cultivated and retain somewhat of their virulence for monkeys and rodents. They are elongated, flexible, corkscrew-like, serpentine and vibratory in

motility, and do not form spores. There is a single terminal flagellum. They are stained with reasonable ease by ptychome methods, especially Giemsa, but not by Gram's method. They measure from 10 to 40 μ in length and about 1 μ in breadth. Coils vary from 6 to 20. The American type is smaller than the rest.

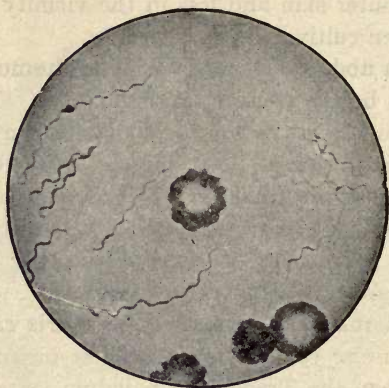


FIG. 77.—Spirilla of relapsing fever from blood of a man. (Kolle and Wassermann.)

Transmission.—The known tick which transmits these organisms becomes infective in one week after biting a patient and remains so all its life; its young are also infective. The types of disease vary but little. In all these is a relapsing fever with periods of apyrexia in between. During the fever the spirochætes are swimming free in the blood and disappear in the afebrile interval.

Cultivation.—They are cultivated in the manner given for *Trep. pallidum* by Noguchi, by adding citrated, therefore defibrinated, blood to serum or ascitic-fluid-fresh-tissue-agar. They breed true to type. They remain alive several days under favorable artificial conditions but cannot be cultivated after

they have left the body a few hours without being on suitable culture media.

The periods of fever last from five to seven days, when a crisis occurs. After an apyrexial period the fever recurs. The spirochætæ are found in great numbers in every microscopical field.

In the apyrexial period the spleen becomes engorged and the leucocytes devour the parasites. Monkeys with excised spleens are more susceptible to infection than others.

Immunity.—The blood from rats that have been immunized by repeated injections of blood from spirochetal rats, if injected into other rats, is capable of conferring an immunity on them by causing spirochætæ to disappear from their blood. One attack seems to confer immunity to the special form causing it but probably not to the others.

SPOROZOA

The most important of this family are the malarial parasites (which belong to the order Hæmosporidia), and the Coccidia.

In general the sporozoa are unicellular organisms that lead a parasite existence in the tissues, especially cells, of higher animals. They ingest liquid food, have no cilia in the adult stage, and flagella are possessed only by the males. There may be one or more nuclei. Propagation is effected by spores, but budding and division do occur, though rarely. Alternate generation takes place frequently.

MALARIAL PARASITES

Hæmosporidia of Man.—The most important disease caused in human beings by the hæmosporidia is malaria, or ague, and excepting the deserts, mountains, and arctic regions, this disease is very widely distributed.

Three different parasites producing different clinical entities are known. According to the time, frequency, and order of the

outbreak of chills and fever, various clinical names have been given to the manifestation of the disease. Mannaberg has arranged the following scheme to show the different forms of outbreaks. The numbers apply to the paroxysms. Each developmental cycle is numbered alike:

I I I I I I I.	<i>Simple quotidian fever.</i>	
I O I O I O I	<i>Simple tertian fever.</i>	
I O O I O O I O O I.	<i>Simple quartan fever.</i>	
I 2 I 2 I 2 I 2.	<i>Double tertian fever.</i>	(Two infections.)
I 2 3 I 2 3 I 2 3.	<i>Triple quartan fever.</i>	(Three infections.)
I 2 O I 2 O I 2 O.	<i>Double quartan fever.</i>	(Two infections.)

The figures refer to days on which paroxysms of fever occur. The o represents the afebrile day.

PLASMODIUM MALARIÆ (*Laveran*)

This is the quartan parasite, and produces in man, in cases of one infection, paroxysms of fever every fourth day.

It appears in the blood, after a paroxysm, as a small non-pigmented body on the bodies of the red blood cells. It has feeble amœboid motion; slowly penetrates the corpuscle, and specks of melanin appear in its protoplasm. Forty-eight hours after the attack the parasite measures from one-half to two-thirds the size of the red cell. Sixty hours after the paroxysm—twelve before the next—the parasite completely fills the red cell, leaving only a narrow rim, which later on disappears. Six hours before the next paroxysm, shizogony begins. The grains of melanin are arranged like the spokes of a wheel, and then, leaving the radii, crowd above the centre (the rest of the cell being pigmentless) gradually dividing into 8 or 12 pear-shaped bodies, or **merozoites**. These separate from each other and individually attack a fresh red cell, and this attack brings another paroxysm of fever seventy-two hours after the previous one. The grains

of pigment are taken up by the leucocytes and deposited in the spleen and bone marrow.

The nucleus of the parasite may be seen if suitably strained. The double or triple quartan is explained by the fact that there are two or three groups of organisms that undergo sporogony at periods separated from each by twenty-four hours.

PLASMODIUM VIVAX (*Grassi*)

The cause of tertian fever occurring in the spring. It differs from the *Plasmodium malariae* because of shorter period (forty-eight hours) consumed in schizogony (or sporulation), the much greater activity of the amœboid movement, and the affected corpuscles becoming enlarged; also by the fact that many of the melanin-bearing stages are visible. The schizogony is rarely apparent in the circulating blood, but in the spleen these stages are easily seen. There are from 15 to 20 merozoites (segmented bodies or spores) which are arranged in an irregular heap, but not radially like wheel spokes. The merozoites are smaller than the quartan variety and are more numerous. The flagellated form can but rarely be seen in the freshly drawn blood. If some blood, containing the large extracorpuseular bodies, is put in a moist chamber, they throw out flagella. These flagella are really *microgametes* and are sexually active. The extracorpuseular bodies are partly *macrogametes*, and if they become flagellated they are called polymites, and are the *microgametocytes*. The merozoites, or spores, finally burst forth from the erythrocytes, starting again another cycle (attended with a paroxysm of fever). These spores appear in the freshly invaded corpuscles as hyaline bodies with slight movement. As they grow in size, pigment appears in the protoplasm. Certain of these do not break up into merozoites, or spores, but become extracellular bodies gametocytes of male or female character. There may be two infections in which schizogony occurs every other day in alternate days, 1 2 1 2 1 2 1 2.

PLASMODIUM FALCIPARUM

The plasmodium of æstivo-autumnal fever, or pernicious malarial fever, also called tropical. The outbreaks of this occur irregularly. The disease produced by them is very much more malignant and is harder to cure. The young spore appears in the corpuscle as a small hyaline body, smaller than the other forms and much more active. The size and shape of the red cells are little if any altered but they become granular and polychromatophilic. The pigment is very finely granular and the body frequently presents the signet-ring appearance. There may be more than one parasite to a red cell. The cycle of development (schizogony) is twenty-four to forty-eight hours. The plasmodium in its schizogony divided into 8 to 24 merozoites or spores, and are arranged in a spore-like form. The extracorporeal bodies may resemble a crescent or sickle; this form is very characteristic of æstivo-autumnal fever. There are two forms of these crescents, one delicate, the male, and one larger and ovoid, the female. They are very resistant to quinine and persist for a long period in the blood. Plasmodia undergoing schizogony are often found in the brain capillaries after death, which accounts for the cerebral symptoms in such cases. This form can be differentiated from the others by the irregular and pernicious type of fever produced; by its great resistance to quinine; the fewer number of merozoites; the finely granular appearance of the pigment; the relatively small size of the young intracorporeal body; and, by the ring shape of some of the young forms.

Often, in blood from malarial cases, pigmented leucocytes are seen, and ghost, or shadow, red corpuscles from which the hæmoglobin has been dissolved are often met with. Spherical extracorporeal bodies become flagellated (gametes) in freshly drawn blood. The parasite may be studied in fresh film preparations and by staining dried films by methylene blue and eosin, Romanowsky's, or Jenner's methods. They are much more frequent in the pyrexial period, and when quinine has not been given.

The various plasmodia are transmitted to man invariably by the anopheles mosquito, in the bodies of which they undergo a different (sexual) existence. It has been positively demonstrated that the various plasmodia undergo an alteration of generations and require two different hosts for their development, *i.e.*, mosquito, man.

The asexual development, or schizogony, takes place in the blood of man, the sporogony, or sexual development, in the body of the anopheles mosquitoes, the bite of which sets up an infection in man, since the sporozoites of the various plasmodia are developed in the salivary glands of these mosquitoes. In the act of biting, the sporozoites reach the erythrocytes where they become the intracorpuseular hyaline bodies beginning again their asexual cycle of development in the blood.

That the mosquito is the intermediate host of the malarial parasite and that the infection in man follows bites by infected mosquitoes has been abundantly proven. The mosquitoes that act in this way are the various *Anopheles*; the *Anopheles maculipennis* being the offender most frequently. The freshly formed schizonts in the blood of an infected man are conveyed into the intestines of the mosquito. Here sexual reproduction of the parasite begins. The male elements, flagellar *microgametes* penetrate the female elements, *macrogametes* (cellular), and after a time there appear intra-cellular fusiform bodies, *ookinets*. These bore into the intestinal walls of the mosquito and there remain. After a time they are converted into round bodies, or *oöcysts*. The nucleus of the *oöcysts* divides rapidly and other daughter nuclei are formed and new cells called *sporoblasts*. After about eight days these form the sporozoites. The number of sporozoites in each *oöcyst* varies from hundreds to many thousands (often 10,000). These *oöcysts* burst and the sporozoites in the circulation find their way to the salivary glands of the mosquito. When a mosquito bites a human being they are introduced into the blood where they are quickly transformed into the intracellular hyaline bodies

DESCRIPTION OF FIG. 78

Life history of malaria parasite, *Plasmodium*. 1, Sporozoite, introduced by mosquito into human blood, the sporozoite becomes a schizont; 2, young schizont; 3, young schizont in a red blood corpuscle; 4, full-grown schizont; 5, nuclear division; 6, spores, or merozoites, from a single mother-cell; 7, young macrogamete (female), from a merozoite, and situated in a red blood corpuscle; 7*a*, young microgametoblast (male); 8, full-grown macrogamete; 8*a*, full-grown microgametoblast; 9, mature macrogamete; 9*a*, mature microgametoblast; 9*b*, resting cell, bearing six flagellate microgametes (male); 10, fertilization of a macrogamete by a motile microgamete; the macrogamete next becomes an ookinete; 11, ookinete, or wandering cell, which penetrates into the wall of the stomach of the mosquito; 12, ookinete in the outer region of the wall of the stomach, *i.e.*, next to the body cavity; 13, young oöcyst, derived from the ookinete; 14, oöcyst, containing sporoblasts, which develop into sporozoites; 15, older oöcyst; 16, mature oöcysts, containing sporozoites; 17, transverse section of salivary gland of an *Anopheles* mosquito, showing sporozoites of the malaria parasite in the gland cells surrounding the central canal.

1-6 illustrate *schizogony* (asexual production of spores); 7-16, *sporogony* (sexual production of spores).

(FOLSOM—After GRASSI and LEUCKART, by permission of Dr. Carl Chun.)

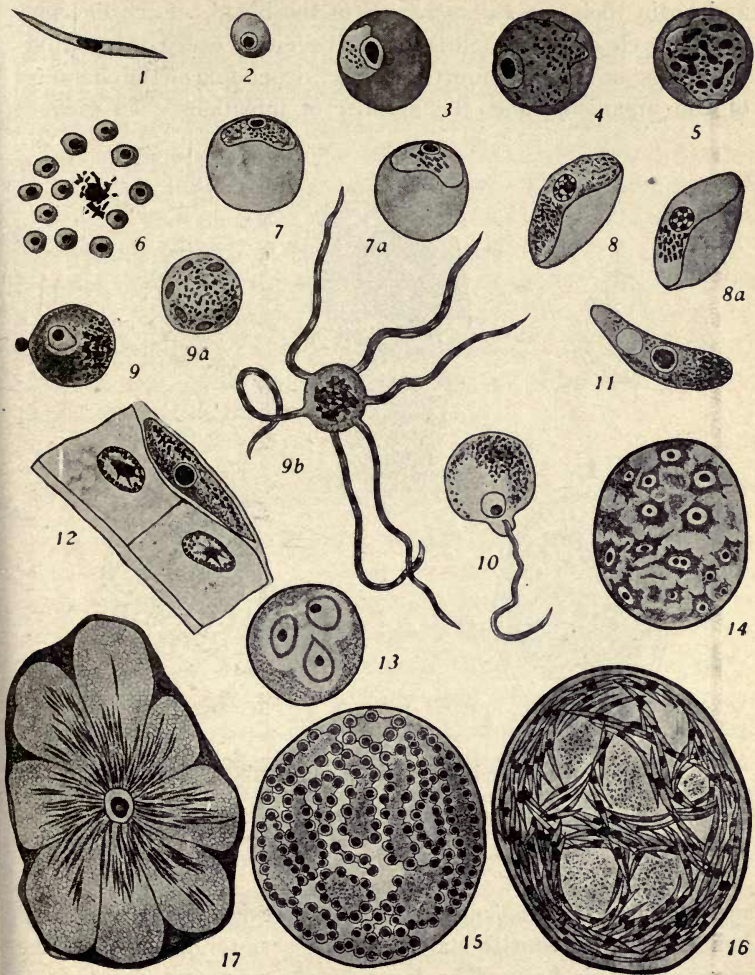


FIG. 78.

and begin their *asexual sporogony* in the blood. Each developmental cycle causing a febrile paroxysm either every day or alternate days, or on every fourth day, etc., depending on the character of the organisms and the number of infections. To prevent

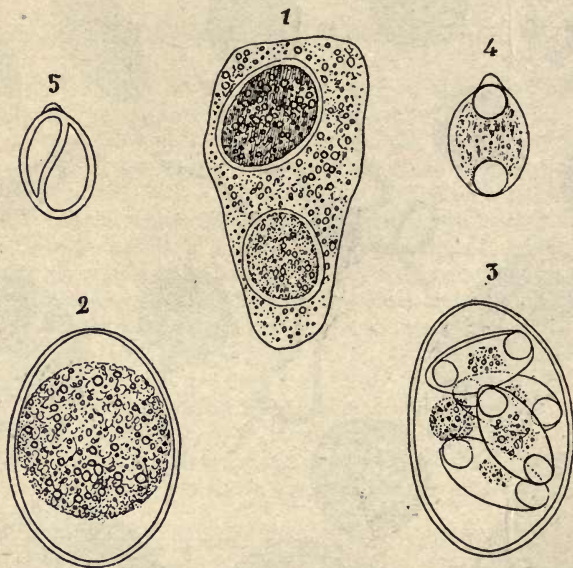


FIG. 79.—*Coccidium hominis*, from intestine of rabbit: 1, a degenerate epithelial cell containing two coccidia; 2, free coccidium from intestinal contents; 3, coccidium with four spores and residual substances; 4, an isolated spore; 5, spore showing the two falciform bodies— $\times 1140$. (From Railliet, in Tyson's Practice.)

spread of malaria, mosquitoes must be prevented from reaching individuals infected with malaria and those not infected. Screens accomplish this best. The larva of the mosquito develops in stagnant water. To prevent the development of these young mosquitoes oil should be poured on the water, thus cutting off the air and means of respiration.

Bass, of New Orleans, claims to have successfully cultivated malarial plasmodia of the species *vivax* and *falciparum* by the use of human blood. He has also succeeded when using Locke's fluid minus calcium chloride plus ascitic fluid. One-half percent dextrose is usually added. The blood is drawn, so that it can be defibrinated, into small flat-bottom tubes. These are incubated at 40°C. The column of fluids is 1-2 inches high, the clear serum layer being $\frac{1}{2}$ inch at least. The parasites grow in the upper layer of the cellular sediment. Undiluted serum and leucocytes are lytic for plasmodia. For renewed cultures these must be removed but uninjured red cells must be added. Only the asexual division has been observed. Leucocytes phagocyte all free parasites under artificial conditions.

COCCIDIUM

Coccidium hominis is another member of the sporozoa that occasionally infects man. Coccidia are infectious also for horses, goats, oxen, sheep, pigs, guinea pigs, weasels and rabbits. The organism is essentially a cell parasite inhabiting the cells of the gastro-intestinal tract by preference, chiefly the liver and intestinal mucous membranes. They lead a sexual and asexual existence like the malarial parasites (alternate generation). The young sickle-shaped nucleated sporozoite penetrates an epithelial cell, where it gradually develops, ultimately dividing into numerous sporozoites. This is the asexual stage of development (schizogony), the sexual stage being called sporogony.

The sporozoites are differentiated into the two sex elements. These are large granular appearing cells; the male being smaller, divides into numerous flagellated microgametes that penetrate the female granular cells, macrogametes, and fertilize them. These fertilized macrogametes, or zygotes, form capsules and become oöcysts which divide into numerous sporoblasts, changing into sickle-shaped sporozoites upon liberation.

The coccidia are easily demonstrable in tissue and in fæces. They produce in man occasionally a fatal disease infecting the liver and intestines. Cattle sometimes die from hæmorrhagic

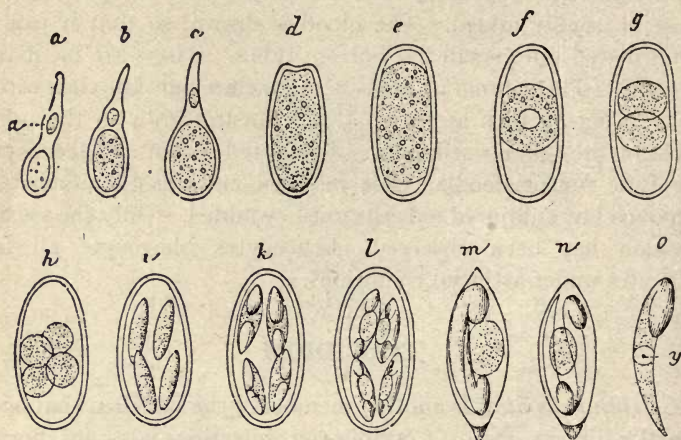


FIG. 80.—Development of *coccidium cuniculi*: *a, b, c*, young coccidia in epithelial cells of gall duct; *d, e, f*, fully grown encysted coccidia; *g, h, i, k, l*, showing development of spores; *m*, isolated spore, greatly magnified, showing the two falciform bodies (*pseudonavicella*; *sporozoites*) in natural position; *n*, a spore compressed so as to separate the two sporozoites, *o*, a sporozoite or falciform body with *y*, its nucleus. (From Railliet after Balbiani—in Tyson's Practice.)

dysentery due to one of the coccidia. The disease is transmitted by the ingestion of food contaminated by fæces containing the sporozoites.

Acid fuchsin stains the sporozoa.

CHAPTER X

THE FILTERABLE VIRUSES

This general term means that the virus of a disease can pass through a porcelain filter and usually that it cannot be seen by the microscope. It, however, does not mean that it is invisible at all stages since in one case at least we have been able by means of the ultramicroscope to see what is almost certainly the particular causal agent. Again it is said that spirochætes when young will traverse porcelain filters. The term will cover in this chapter those diseases of importance to man whose causal agents cannot be morphologically described, but whose characters are more or less well known. The list of diseases caused by submicroscopic agents is as follows: African horse sickness, swamp fever of horses, catarrhal fever of sheep, yellow fever, Dengue, three-day fever, typhus fever, poliomyelitis, rabies, variola, with its congeners vaccinia and animal pox, hog cholera, foot and mouth disease, fowl plague, fowl diphtheria, transplantable sarcoma and leukemia of fowls, cattle plague, trachoma, pleuropneumonia of cattle, mollusum contagiosum, measles, scarlet fever, guinea-pigs epizootic and some diseases of plants. As said above, only the diseases transmissible to human beings are reviewed.

Some of the above diseases, notably rabies, scarlatina and trachoma, show in the leucocytes and epidermal cells certain structures or inclusion bodies to which the name Chlamydozoa was given by Prowaczek, a term implying that a parasitic body is growing in a mantle. They start as tiny specks in the cytoplasm shortly found to be surrounded by a clear, sharply outlined halo. They seem to grow at the expense of the host cell. Their exact character is not understood; they are probably evidences of cellular degeneration under the influence of some noxa.

Hydrophobia.—This disease has long been considered to be an infectious one, but the causal parasitic agent has never been discovered. It is commonly found in dogs, cats, wolves, rabbits, etc., but other domestic animals, and man may become infected. It is a disease of the central nervous system, highly infectious, always following a bite or other injury in which the skin is broken, and in which lesion the virus may be deposited. Infection may be caused by injecting emulsified infected nerve tissue (brain) into susceptible animals (rabbits or monkeys). The disease is always fatal after it is well established. Well-marked histological lesions of the central nerve tissues, particularly the large ganglia, have been found by Van Gehuchten and Nelis, and Ravenel and McCarthy. If emulsified brain tissue from an animal that has died of hydrophobia is filtered through a "germ-proof" filter the filtrate is capable of setting up the disease in a healthy animal if it is injected into it. By long centrifugation of emulsified infected brain tissue, the supernatant fluid loses its power of reproducing the disease on injection. Virus may also be found in mammary and lacrymal secretions, pancreas, cerebro-spinal fluid and aqueous humor.

The organism is toxic in character, since filtrates sometimes fail to produce transmissible disease, but emaciation, paralysis, and death are caused by their injection into rabbits, the tissues of which, in turn, are not infectious.

The unknown organisms are rather resistant to agents that are germicidal. They are destroyed in fifty minutes by a 5 percent carbolic solution, and in three hours by a 1-1,000 corrosive sublimate solution. Direct sunlight kills them quickly, as do radium emanations. The latter have been used as curative measure with reputed success. A temperature from 52° to 58°C. for one-half hour destroys them, but they resist extreme cold of liquid air (-312°) for many weeks. Pasteur found that desiccation attenuated the virus. Chlorine kills it quickly, while glycerine does not. The virus may be increased in virulence by passing the "street virus" of dogs through a series of rabbits. Here the period of

incubation decreases from three weeks to six days, but beyond this the period does not become less, and the degree of virulence from the virus lead Pasteur to name it *virus fixe* (fixed virus).

Passing the virus through foxes, cats, and wolves also intensifies the virulence, while monkeys and chickens attenuate it.

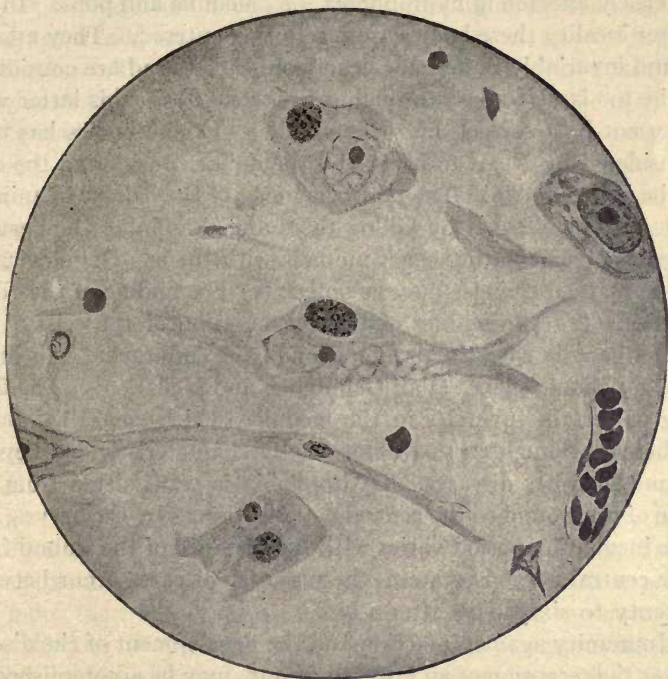


FIG. 81.—Section through the *cornu ammonis* of brain of a rabid dog; stained by the method of Lentz. Five Negri bodies of different sizes are shown, enclosed within the ganglion cells. The smallest contains only three minute granules. (After Lentz, *Centralbl. f. Bakt.*, 1907, Abt. I, Vol. XLIV, p. 378.)

Negri bodies, intracellular bodies discovered by Negri, are found in the ganglionic cells of rabid animals. These bodies stain by eosin, and are from 1 to 27μ in size, being generally about 5μ .

They are found particularly in the cornu of Ammon; in Purkinje's cells in the cerebellum; and in the larger cells of the cortex of the cerebrum. These may be the cause of the disease, but there are several objections to this hypothesis. Their distribution does not correspond to the parts of the nervous system that are most intensely affected in hydrophobia, *i.e.*, medulla and pons. In the latter locality these bodies are rarely encountered. They are not found invariably in animals dead from rabies, and are considered to be too large to pass through a Berkefeld filter; this latter view may not be a correct one. The finding of these bodies has been considered by Negri to be good grounds for considering the case to be hydrophobia. The rapid diagnosis of the disease in animals can only be effected by killing them and examining the nervous tissues, or inoculating other animals with them. Histologically, three marked changes may be noted: (1) The finding of the Negri bodies. (2) The finding of the degeneration of the cells of the larger ganglia with the proliferation of the endothelial cells lining the ganglionic spaces (Van Gehuchten and Nelis). (3) The finding of certain tubercles in the medulla, which are called Babes tubercles, though these are not wholly characteristic, as they are found in other diseases. Hydrophobia is transmitted from the site of the wound to the central nervous tissues by the nerves, and the incubation period varies with the distance of the wound from the central nervous system; the majority of cases occur between twenty to sixty days after a bite.

Immunity against infection and the development of the disease after the reception of an infected wound, may be accomplished by Pasteur's method (see chapter on Vaccine).

Yellow Fever

That this disease is caused by a parasite there can be no doubt. It is highly infectious and largely confined to the tropical regions of the western hemisphere and in parts of Africa. Resembling diseases established as due to protozoa, this one is unquestion-

ably spread by mosquitoes, and it has been definitely determined by Carrol and Reed that the female *Aedes capolus* (formerly called *Stegomyia fasciata* and *St. calopus*) is the means of its propagation. Carrol believes that the undiscovered parasite of yellow fever is of the animal kingdom, for the following reasons: (1) It is absolutely necessary for its continued existence that it undergoes *alternate generation* in man and in the *Stegomyia* mosquito. This is peculiar to the sporozoa. (2) The fact that twelve days must elapse before the mosquito is capable of infecting man is evidence that a cycle of development of the unknown parasite is taking place in the mosquito. (3) The limitation of the cycle of development of the parasites to a single genus of the mosquito and to a single vertebrate (man) conforms to a natural zoologic law, and this does not conform to our knowledge of the life history of bacteria. (4) The effects of climate and temperature on the life history of the *stegomyia*, and on the rate of development of the parasites in the bodies of the mosquitoes are exactly the same as the effects of the same conditions on the *anopheles* mosquito and the malarial parasite. Without the *stegomyia* there can be no yellow fever. Infection requires the fulfilling of the following conditions: (1) By the bite of the mosquito providing the insect has fed on the blood of a yellow fever patient within the first three days of the fever. (2) The disease is not transferred immediately, but a definite incubative period of more than eleven days must elapse before the mosquito can transfer the disease. After twelve days the mosquito has been found to be infected for at least fifty-seven days. (3) Yellow fever cannot be carried by fomites. (4) Yellow fever may be produced in a healthy man by the subcutaneous injection of blood from a yellow fever case (parasites in the blood). (5) The serum of a yellow fever patient filtered through a very fine Berkefeld or porcelain filter is still capable of setting up the disease if injected, proving that the infection agent is capable at some stage of its life to pass through filter pores. (6) An attack of yellow fever

produced by the bite of a mosquito confers immunity against subsequent infection. (7) The period of infection is usually three days but may be from two to six days. (8) A house or ship may be said to be infected with yellow fever only when there are present mosquitoes capable of conveying the parasite of the disease. (9) The spread of yellow fever may be prevented by destroying the aedes and preventing egress and ingress of the insects from yellow fever patients to the non-immune. (10) No insect, other than the aedes, has been found to be concerned in the spread of yellow fever.

Noguchi reports the discovery of a tiny spirochæte, $4-9\mu$ long and $.2\mu$ wide, in the blood of yellow fever patients. The organism may be transferred to guinea pigs in which it produces lesions comparable to those of human yellow fever. It may be cultivated in anaerobic serum water tubes and it will pass through a filter. During an attack, either human or artificial in a guinea pig, protective anti-bodies are formed. Spirals are only found in the blood in the first few days of an attack. They do not stain well and can best be seen by aid of the dark field microscope. It is claimed that vaccination with these spirals produces some resistance to yellow fever.

Yellow fever is a tropical or subtropical disease, because the aedes is confined to these regions, and the disease is found in low moist localities rather than those that are drier and higher, from the fact that the mosquito inhabits the former and not the latter. Yellow fever dies out after the first sharp frost, because the aedes are then either killed or undergo hibernation. Many conclusive experiments by Reed and Carrol, by Guiteras, and by the French Commission have proved that the aedes is beyond doubt the cause of the spread of the disease. No immunity, other than the activity acquired one, is known.

Small-pox and Vaccinia.—These two diseases must be considered to be but two clinical activities of one unknown specific microörganism.

Certain protozoonoid bodies have been seen by numerous observers, notably by VanderLoeff, L. Peiffer, and Guarnieri. The latter gave the name *Cytoryctes vacciniæ s. variolæ*. In the deep layers of the epithelial cells of the pustules of vaccinia and small-pox, in the experimental lesions on the corneæ of rabbits, and in the protoplasm of the cells, these bodies are found. They are about the size of a micrococcus and exhibit amœboid movements in hanging-drop preparations. They are perfectly characteristic of the lesion produced in vaccinia and are not found in other diseased conditions.

In variola many different changes occur in the appearances of these cytoryctes, suggesting developmental cycles.

In variola they are often intranuclear, while in vaccine they are never found within the nuclei.

The cycle of development is suggestive of the development of many of the protozoa. Stages of development exhibiting fusiform amœboid shapes can be seen, and pseudopodia can be detected in the process of developmental stages suggestive of gametocytes; the union of the gametes and the ultimate formation of the zygote can also be discerned.

After the tenth day these bodies cannot be very well discerned in the tissues.

There is reason to think that the parasites circulate in the blood in variola. The contagion in variola is thought to be by inhalation. It is certain that the disease can be produced by inoculation with virus from a case of small-pox. The contagion exists in the scales, pus cells, and excretions of patients ill with small-pox.

If the virus of small-pox is introduced into a monkey, and then into a cow the disease produced is not variola, but vaccinia (Monkman). The hypothetical organism above described, *cytoryctes*, becomes attenuated in the cow, so that it is incapable of producing variola, but vaccinia.

Rabbits, horses, and sheep are susceptible of inoculation with the virus of vaccinia (see Vaccination). Virus may be tested by

rubbing over the shaven bellies of rabbits, setting up minute vesicles and finally crusts (Calmette).

The two viruses, that of variola and that of vaccinia, are now thought to be identical. In a diluted condition it is filterable. It resists drying for weeks and glycerine eight to ten months. It is destroyed at 57°C. in fifteen minutes and easily by most disinfectants. Passive immunization has not been achieved. Noguchi has succeeded in growing the virus in the testes of rabbits; one of his objects in so doing was to afford a sterile cultivation of virus which might be used for preparing vaccine.

Scarlet Fever

Mallory in 1903 found certain bodies in the skin of scarlet fever cases. These bodies, he assumed, were protozoan in character and were the etiological cause of the disease. He named them **Cyclasterion Scarlatinale**. They have been found rather constantly in the skin of scarlet fever cases, also in the skin in cases of measles and in anti-toxin rashes. Döhle has also described an inclusion body within the polynuclear leucocytes. By several observers these bodies have been considered to be artefacts or degeneration products in the epithelial cells.

The virus of scarlatina is now considered to be filterable and transmissible to monkeys. Mallory has lately found diphtheroid bacilli in great numbers in the pharynx in scarlatina and believes they may be its cause. Virulent streptococci, usually of hemolytic quality, are so frequently encountered in the respiratory tract and in complications during scarlet fever that many persons look upon them as the cause; this cannot be proven as the disease cannot be successfully transmitted to lower animals by use of these organisms.

Dengue Fever.—This is an acute infectious disease of the tropics, characterized by fever, skin eruptions, rheumatoid pains, an afebrile remission and a febrile end, due to a filterable virus,

transmitted by the mosquito, *Culex fagitans*. The virus is in the blood-stream. One attack gives immunity; little is known of the virus.

Three-day or Sand-fly Fever.—A mild infectious disease chiefly of southeastern Europe, due to a virus which will pass through a bacteria-proof filter and is transmitted by the sand-fly, *Phlebotomus pappatacii*. Cultures have not been obtained.

Typhus Fever or Spotted Fever.—An acute epidemic disease with prolonged course, prostration, a macular eruption, ending by crisis, transmitted by the body louse, *Pediculus vestimenti*. The virus is filterable but is obtained with difficulty. It is found best toward the end of the fever. It may be transmitted to monkeys. It is destroyed quickly at 52°C. Brill's disease is a mild typhus fever. A Gram-positive anaerobic non-motile bacillus $.2-.6 \times .9-2\mu$, has been described as inhabiting the blood in typhus. As claims to being the cause of the disease one finds that injection causes a distinct febrile reaction in guinea pigs and the culture may be used as antigen in a complement fixation test.

Poliomyelitis.—An acute infectious disease, chiefly of children characterized by a short febrile attack, followed by a rapidly appearing paralysis in various muscles. Means of transmission from child to child is unknown, but it has lately been shown that the stable fly, *Stomoxys calcitrans*, can transmit it from monkey to monkey. Greater weight is, however, laid upon transmission from person to person by contact and the emanations from the upper respiratory; this idea gains in value because monkeys can be infected by nasal washings from patients during the disease. The virus is in the central nervous system, lymphatic system, blood, succus entericus, nasal mucous and various organs. It is said to be constantly in the nasal mucosa of not only patients but of the well in their vicinity. This is supposed to be its portal of entry to the body. It is transmitted to monkeys by injecting emulsions of the virus-containing parts into the brain, blood-stream or peritoneum. It can be filtered through porcelain. It resists

glycerine, drying and autolysis. It is destroyed at 50°C. in one-half hour. The virus can be cultivated by growing bits of brain and cord from a case dead of the disease in unheated ascitic fluid to which bits of sterile tissue have been added; the growth is anaerobic. It appears as tiny globoid bodies, singly, in pairs or short chains. Appropriate staining will demonstrate them also in the nervous tissues.

Active artificial immunity and some passive immunity have been obtained but these are not of therapeutic value. One attack probably confers immunity. In the therapeutics of the disease it is practicable to inject into the spinal column, the blood serum of human cases that have recovered from the disease; this indicates that virus-neutralizing bodies are formed during an attack.

Foot and Mouth Disease.—An acute infectious disease of cattle, characterized by a vesicular eruption in the mouth and around the crown of the hoof. It may be transmitted to man by the use of milk from infected cows. It is also directly communicable. It has not been cultivated. It is filterable; it is said to be due to the Cytorrycetes. It is destroyed at 50°C. in ten minutes, easily by freezing and ordinary disinfectants. One attack gives no lasting immunity but the blood is said to contain anti-bodies immediately after the attack, which will be protective to other animals.

Trachoma.—An infectious inflammation of the conjunctiva with the production of minute but visible nodules on the under sides of the lids. By some it is said to be due to a form of the influenza bacillus, by others to an invisible virus. It is directly communicable, filterable and transmissible to monkeys. It has not been cultivated.

Measles.—An acute eruptive fever due to a filterable virus which is found in the blood, buccal and nasal secretions. It is transmissible to monkeys by inoculations of patient's blood, even before the Koplik spots appear. It persists in the blood until

after the appearance of the eruption. It resists drying and freezing. It is destroyed at 55°C , in fifteen minutes; it has not been cultivated. Immunity follows an attack but no passive immunity has been reported.

Mumps.—This disease seems also to be due to a filterable virus, resident within the parotid gland, capable of transmission to monkeys. Many organisms have been described but none is probably specific.

It must be said of both the hypothetical organisms of variola and scarlatina, that if they are the cause of these two diseases they differ from all other known protozoan parasites, because the latter require an intermediate host for the transmission of the parasite from individual to individual while these certainly do not.

Rocky Mountain Fever is not due to a filterable virus but its agent resides in the blood. It is a disease of the Rocky Mountain States characterized by general pains; macular eruption and constitutional symptoms of infection, it is transmitted by means of the tick, *Dermacentor venustus*. The female tick obtains the agent by blood sucking and can transmit it to her young. The virus is destroyed by heating and drying. A minute coccoid body has been found in the tick but it has not been cultivated. One artificial attack in the guinea pig leaves immunity, as does the spontaneous disease in man.

Trench Fever is a disease which became known in the Great War characterized by fever, pains in the legs, back and head, with marked dizziness, due to a resistant filterable virus, in all probability transmitted by lice; the virus is found in the blood. The entrance is effected either by the bite of a louse or its feces may be scratched into tiny wounds on the skin. The virus seems to be excreted in the urine and sputum. It is not killed at 80°C . The louse is not infective for seven days after biting a patient and then remains infective for three weeks. No immunity follows an attack.

Encephalitis lethargica is a disease alleged to be due to a virus. It appears sometimes in epidemic form and was observed after the last pandemic of influenza; some persons believe the two are related. Some students of the subject report successful transmission to monkeys.

TABLE
Representing the Important Morphological and Chemical Characteristics of Bacteria
(Arranged from Lehman & Neumann.)

Signs + indicates presence.
 — indicates absence.
 Δ indicates presence at times and absence at times.

Name	Flagella	Gram's Stain	Aerobic and Anaerobic Growth		Liquefaction of Gelatine	Buoillon culture		Milk Culture		Spore-formation	Indol Reaction	Production of Gas in Sugar-agar
			Aerobic	Anaerobic		Pellicle	Cloudiness	Coagulation	Reaction			
<i>Streptoc. pyogenes</i> ...	—	++	++	++	—	—	—	++	Acid	—	—	—
<i>Streptoc. lanceolatus</i> .	—	++	++	++	—	—	—	++	Acid	—	—	—
<i>Streptoc. meningitidis</i>	—	++	++	++	—	—	—	Δ	Faintly alkal	—	—	—
<i>Mic. tetragenus</i>	—	++	++	++	—	—	Marked	+	Acid	Very slight	—	—
<i>Mic. pyogenes</i> <i>a</i>	—	++	++	++	—	—	—	+	Acid	—	—	—
<i>Mic. pyogenes</i> <i>β</i>	—	+	+	+	—	—	—	—	Acid	—	—	—
<i>Mic. pyogenes γ albus</i>	—	++	++	+	—	—	Slight	Δ	Faintly acid	A trace	—	—
<i>Mic. gonorrhoeæ</i>	—	—	—	—	—	+	+	—	— Alkaline	—	—	—
<i>Mic. melitensis</i>	—	—	—	—	—	+	Cloudy at first	—	—	—	—	—
<i>Bact. pestis</i>	—	—	—	—	—	+	—	—	—	—	—	—

Name	Flagella	Gram's Stain	Aerobic and Anaerobic Growth		Liquefaction of Gelatine	Bouillon Culture		Milk Culture		Spore-formation	Indol Reaction	Production of Gas in Sugar-agar
			Aerobic	Anaerobic		Pellicle	Cloudiness	Coagulation	Reaction			
Bact. influenzae.....	—	—	+	+	—	—	Moderate	—	Acid	—	Slight	—
Bact. pneumoniae....	Many	—	+	+	—	—	Slight	—	Amphoteric	—	—	—
Bact. typhosus.....	A few	—	+	+	—	—	Moderate	—	Acid	—	Pronounced	—
Bact. coli.....	Many	—	+	+	—	—	Marked	+	Acid	—	Very slight	—
Bact. prodigiosum....	—	—	+	+	—	—	+	+	+Acid	—	+	—
Bact. dysenteriae.....	A few	—	+	+	—	—	Moderate	+	Acid	—	—	—
Bact. violaceum.....	+8	—	+	+	—	—	+	+	Amphoteric	—	—	—
Bact. enteritidis.....	One	—	+	+	—	—	Marked	+	later alka.	—	—	—
Bact. pyocyaneum....	Many	—	+	+	—	—	Very slight	—	Faintly alkal.	—	Slight	—
Bact. Zopfi.....	Many	+	+	+	—	—	Moderate	+	Faintly acid	—	Very pronounced	—
Bact. vulgare.....	Many	+	+	+	—	—	Moderate	—	Faintly alkaline	—	Faint without nitrite	—
Bact. vulgare β	—	+	+	+	—	—	Moderate	—	Amphoteric	—	—	—
Bact. mirabilis.	—	+	+	+	—	—	Slight	+	Faintly alkaline	—	—	—
Bact. erysipelatosuum.	—	+	+	+	—	—	Very marked	+	Alkaline	—	—	—
Bac. anthracis.....	—	+	+	+	—	—	Moderate	+	+Acid	—	—	—
Bac. mycoides.....	—	+	+	+	—	—	+	+	—	—	—	—
Bac. Botulinus.....	+	+	+	+	—	—	+	+	—	—	—	—

Name	Flagella	Gram's Stain	Aerobic and Anaerobic Growth		Liquefaction of Gelatine	Bouillon Culture		Milk Culture		Spore-formation	Indol Reaction	Production of Gas in Sugar-agar
			Aerobic	Anaerobic		Pellicle	Cloudiness	Coagulation	Reaction			
Bac. cap. aerogenes...	—	++	+	++	+	++	+	++	Acid faintly alkaline	++	—	+
Bac. subtilis.....	Many	++	+	++	+	++	Very marked	++	Faintly alkaline	++	—	+
Bac. megatherium...	Many	++	++	++	++	++	Moderate	+	Strongly alk.	++	—	—
Bac. vulgatus.....	Many	++	++	++	++	++	Moderate	Δ	Faintly alk.	++	—	—
Bac. mesentericus...	Many	++	++	++	++	++	Moderate	+	Amphoteric	++	A trace	—
Bac. tetani.....	Many	++	++	++	++	++	Moderate	+	Faintly acid	++	Slight	—
Bac. Chauvœi.....	Many	++	++	++	++	++	Moderate	+	Amphoteric	++	Slight	—
Bac. œdematis maligni.	Many	++	++	++	++	++	Moderate	+	Amphoteric	++	Slight	—
Vibrio cholerae.....	One, seldom Two	+	+	+	+	+	Moderate	+	Acid	+	Pronounced without nitrite	—
Vibrio proteus.....	One	++	++	++	++	++	Moderate	+	Acid	+	Slight	—
Spir. rubrum.....	A bunch	++	Δ	++	++	++	Slight	+	Faintly alk.	+	A trace	—
Corynebact. mallei...	—	++	++	++	++	++	Moderate	Δ	Acid	+	Slight	—
Corynebact. diphtheriæ.	—	++	++	++	++	++	Very slight	Δ	Acid to alk.	+	Slight	—
Coryneb. pseudodiphtheritic.	—	+	+	+	+	+	Almost clear	+	Faintly acid	+	Slight	—
Corynebact. xerosis...	—	+	++	++	++	+	Clear	+	Amphoteric	+	—	—
Mycobact. tuberculosis.	—	X	++	++	++	++	Clear	+	Amphoteric	+	—	—
Mycobact. lepræ....	—	++	++	+	+	+	—	+	Faintly alk.	+	A trace	—
Actinomyces bovis...	—	++	++	+	+	+	—	+	Faintly alk.	+	A trace	—

DESCRIPTION OF PLATE I

Malarial Parasites

1. Two tertian parasites about thirty-six hours old, within blood corpuscles magnified.

2. Tertian parasite about thirty-six hours old; stained by Romanowsky's method. The black granule in the parasite is not pigment but chromatin. Next to it and to the left is a large lymphocyte, and under it the black spot is a blood plate.

3. Tertian parasite division form nearby is a polynuclear leucocyte.

4. Quartan parasite, ribbon form.

5. Quartan parasite, undergoing division.

6. Tropical fever parasite (æstivo-autumnal). In one blood corpuscle may be seen a smaller, medium, and large tropical fever-ring parasite.

7. Tropical fever parasite. Gametes half-moon spherical form. Smear from bone marrow

8. Tropical fever parasite which is preparing for division heaped up in the blood capillaries of the brain.

Asexual Forms

9. Smaller tertian ring about twelve hours old.

10. Tertian parasite about thirty-six hours old, so-called amœboid form.

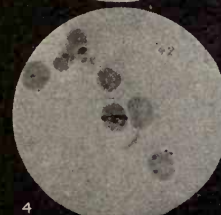
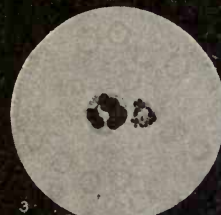
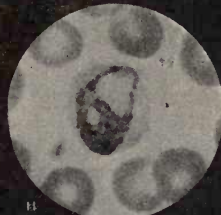
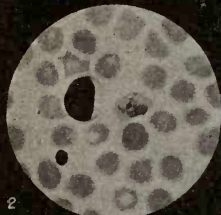
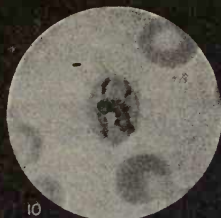
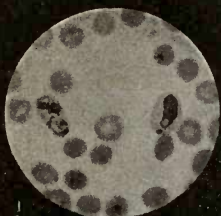
11. Tertian parasite still showing ring form, forty-two hours old.

12. Tertian parasite, two hours before febrile attack. The pigment is beginning to arrange itself in streaks or lines.

13. Tertian parasite further advanced in division. Pigment collected in large quantities.

14. Further advanced in the division (tertian parasite).

PLATE I

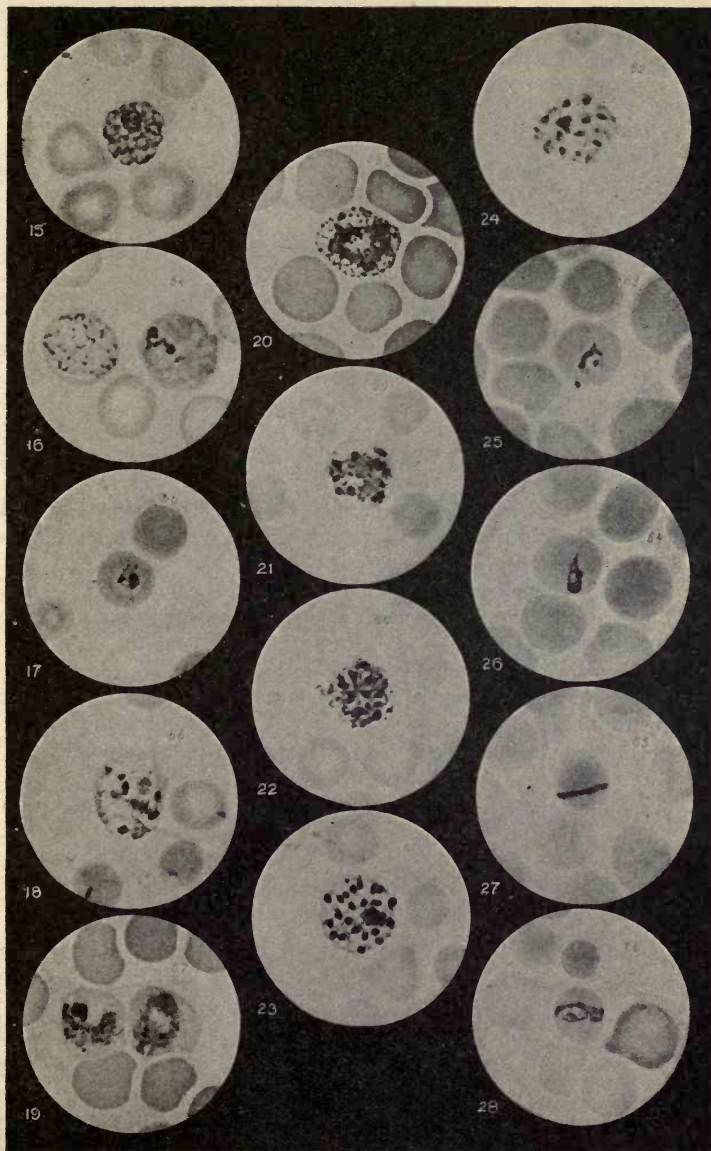


DESCRIPTION OF PLATE II

Malarial Parasites

15. Complete division of the parasite. Typical mulberry form.
16. To the left is the completed division form, an almost developed gamete which is to be recognized by its dispersed pigment.
17. A tertian ring parasite, small size broken up.
18. Three-fold infection with tertian parasite. The oval black granules are the chromatin granules.
19. To the left, tertian parasite with large, sharply demarked, and deeply colored chromatin granules. To the right, tertian parasite. Both thirty-six hours old. Both probably gametes.
20. Tertian parasite thirty-six hours old, ring form.
21. Tertian parasite with beginning chromatin division, with eight chromatin segments.
22. Tertian parasite chromatin division farther advanced with twelve chromatin granules, in part triangular in form.
23. Completed division figure of a tertian parasite. Twenty-two chromatin granules.
24. The young tertian parasites separating themselves from each other. The pigment remains behind in the middle.
25. Quartan ring parasite, which is hard to differentiate from large tropical ring or small tertian ring.
26. Quartan ring lengthening itself.
27. Small quartan ribbon form.
28. The quartan ribbon increases in width. The dark places consist almost entirely of pigment.

PLATE II



DESCRIPTION OF PLATE III

Malarial Parasite

29, 30, 31. The quartan ribbon increases in width. The dark places consist almost entirely of pigment.

32. Beginning division of the quartan parasite and the black spot in the middle is the collected pigment.

33. Quartan ring.

34. Double infection with quartan parasites.

35. Wide quartan band. The fine black stippling in the upper half of the parasite is pigment.

36. Beginning division of the quartan parasite. The chromatin (black fleck) is split into four parts.

37. Division advanced, quartan parasites.

38. Typical division figure of the quartan parasite.

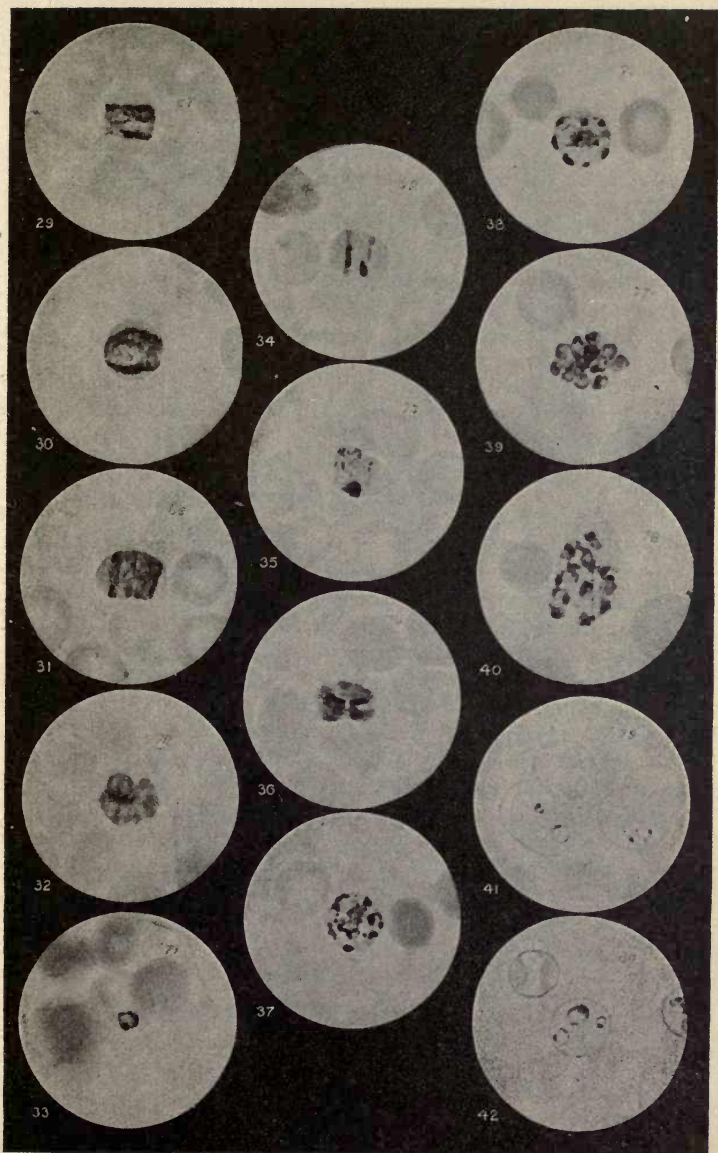
39. Finished division of the quartan parasite. Ten young parasites, pigment in the middle.

40. Young parasites separated from one another.

41. Small and medium tropical ring, the latter in a transition stage to a large tropical ring.

42. Small, medium and large tropical ring, together in one corpuscle.

PLATE III



DESCRIPTION OF PLATE IV

Malarial Parasite

43. To the left a young (spore) tropical parasite. To the right a medium and large tropical parasite.

44. An almost fully developed tropical parasite. The black granules are pigment heaps.

45. Young parasites separated from one another. Broken up division forms twenty-one new parasites.

46. To the left a red corpuscle with basophilic, karyochromatophilic granules. Prototype of malarial parasite. On the right a red blood corpuscle with remains of nucleus.

Sexual Forms or Gametes

47. An earlier quartan gamete (macrogametocyte in sphere form), female.

48. An earlier quartan gamete (microgametocyte), male.

49. Tertian gamete, male form (microgametocyte).

50. Tertian gamete, female (macrogamete).

51. Tertian gamete (macrogametocyte still within a red blood corpuscle.

52. Microgamete tertian within a red blood corpuscle.

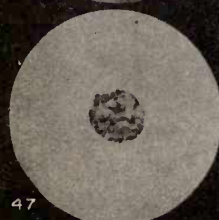
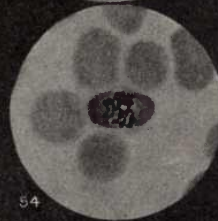
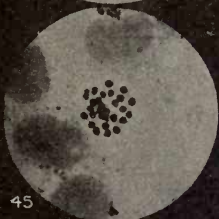
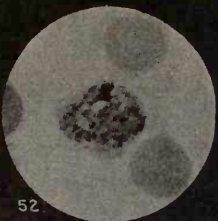
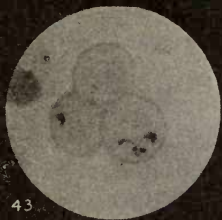
53. Tropical fever. (Æstivo-autumnal) gamete, half moon (crescent) still lying in a red blood corpuscle. In the middle is the pigment. The concave side of the crescent is spanned by the border of the red blood corpuscle.

54. Gamete, tropical fever parasite.

55. Gamete of tropical fever parasite heavily pigmented.

56. Gamete of the tropical fever parasite (flagellated form), microgametocyte sending out microgametes (flagella or spermatozoon).

PLATE IV



CHAPTER XI

BACTERIOLOGY OF WATER, AIR, SOIL, AND MILK

Bacteriological examination of water is of importance for the determination of the presence of pathogenic bacteria, and for the enumeration of the total number of all bacteria contained therein, the latter being considered an index of the purity of the water.

Several well-known pathogenic bacteria have been found in water; among these are the typhoid, anthrax, cholera, plague, and colon bacilli, also the pus cocci. Since the tetanus bacillus is a normal inhabitant of the cultivated soil and manure, it is not at all uncommon to find it, at times, in muddy waters.

Bacteriological examinations of water are, in a measure, very disappointing, because it is difficult, and at times impossible to determine the presence of the typhoid bacillus, even when it is certain that it is present; having been added to water to be examined it is even then difficult to isolate.

The fact that the colon bacillus is always found in water contaminated by fæces is a great help in the recognition of polluted water. In the case of typhoid contamination the typhoid bacillus may elude detection, but the colon bacillus is easily found; we may then assume that, since it is impossible for typhoid bacilli to reach water without the colon bacilli that water having no colon bacilli is also free from typhoid bacilli. Also water having colon bacilli in great numbers is contaminated with fæces, and perhaps typhoid fæces. The detection of the colon bacillus is therefore of prime importance in the examination of drinking water. Its detection is simple. Water must be collected in sterile bottles, using every precaution against accidental contamination. Fermentation tubes are employed, containing bouillon with 1 percent of lactose. Into a series of these tubes,

varying amounts of water are run by means of a sterile pipette, 2 c.c., 1 c.c., .5 c.c., .1 c.c., .01 c.c., of water being used. After a stay of twenty-four hours in the incubator, if gas appears, the bouillon should be examined by plate cultures for the colon bacillus. Lactose litmus agar is used, and where colonies appear that redden the litmus and resemble the colon colonies in appearance, they are planted in milk, fermentation tubes, peptone solution, neutral red agar, nitrate solution, and gelatine, and the various reactions in the various media noted. Some idea of the numerical presence of colon bacilli can also be obtained. Definite quantities of the raw water, similar to those used in the fermentation tubes, may be plated directly without previous incubation. A deeply tinted litmus lactose agar is used and upon this medium colon bacillus colonies appear, small, pink, round or whetstone-shaped surrounded by a pink zone or halo. Such pink colonies are fished out into the different media as above. If there were twenty pink colonies of the colon type upon a plate of litmus lactose agar that had been seeded with 1 c.c. of water and of these eight were fished and determined, with the discovery that four only were true *B. coli*, we would assume that in 1 c.c. of raw water half the pink growing colonies were those of *B. coli* and that the water contained ten *B. coli* per cubic centimeter.

The significance of the colon bacilli is often overestimated. They are found in all rivers, and often reach streams, wells, and even springs by contamination from the barnyard, or manured fields. Attempts to separate colon bacilli from human and animal sources have been unsuccessful. Some authorities use streptococci of the faecal type as pollution indicators. This is not absolutely reliable.

Typhoid bacilli have been found in water. One way that is sometimes successful is to take 25 c.c. of a 4 percent peptone solution and add this to a litre of the water to be examined; from this, after twenty-four hours in an incubator, plates may be prepared with the agar medium of Endo as already given on page 120.

To Count Bacteria in Water

The sample must be collected in a sterile bottle, and the plates poured immediately, since bacteria multiply enormously after a few hours.

Take $\frac{1}{10}$ c.c. or $\frac{1}{2}$ c.c. or 1 c.c. of the water in sterile pipettes and mix with a tube of melted gelatine or agar, pour quickly into cool sterile petri dishes and place in a cool dry place. The American Public Health Association also recommends the use of + 1 percent agar plates grown both at room and body temperature. The counts for the two are averaged. After forty-eight hours count the colonies and the result (after multiplication where $\frac{1}{10}$ or $\frac{1}{2}$ c.c. of water was used) will be the number of bacteria per cubic centimeter. It may be necessary to dilute the water five or ten times before pouring plates. A glass plate ruled into squares, known as a Wolffhügel plate, should be used for counting. The number of bacteria in potable waters varies in many ways, according to the amount of pollution, or albuminous matter in the water, while depth, and the swiftness with which it flows are conditions that modify bacterial contents. The water in a reservoir becomes almost free from bacteria during the first ten days. The number of bacteria diminishes 10 percent per day for the first five or eight days, due no doubt to gravitation of the bacteria to the bottom, also in part to the action of light, which plays an important rôle in the destruction of the bacteria of water supplies.

In general, water containing less than 100 bacteria per cubic centimeter is considered to be from a deep source, and uncontaminated by drainage. Deep artesian wells often contain but from 5 to 15 bacteria per cubic centimeter, water from rivers often contain 12,000 or 20,000 depending somewhat upon the season of the year. Rains cause an augmentation of the bacterial content. Summer causes a diminution.

In identifying a certain water supply as the cause of an epi-

demic of typhoid, the number of bacteria is of great value in locating the place of infection.

The efficiency of filters in large municipal water supplies is known only by the bacterial content of the effluent. In good sand and mechanical (alum) filters, the reduction in the number of bacteria is often over 95 percent (sometimes 99 percent). Plate cultures should be made daily from every filter in order to determine how each filter is performing. Sand filters should not filter more than 1,000,000 gallons per acre a day. They should be at least 1 metre thick; the upper $\frac{1}{2}$ inch of the sand performs over 90 percent of the filtration, due to a certain zooglea, or growth of bacteria. Cracks, or imperfections in the filter beds are quickly detected by the rapid increase of the number of the bacteria in the effluent. It is supposed that not only are bacteria filtered by the sand but that destructive changes occur in the filter which greatly diminish the number of bacteria. A filter must be used for a few days before it becomes efficient or "ripe." After a time it becomes inefficient and it must then be scraped, finally the sand must be removed and washed.

A sand filter is a highly efficient means of water purification. It often converts a foul dirty water into a bright, clean, wholesome water of low bacterial content.

Mechanical filters depend for their efficiency upon the addition of aluminum sulphate to the water. This is decomposed by the carbonates and aluminum hydroxide is produced, which is a white jelly-like flocculent precipitate, which mechanically entangles bacteria and removes them from the water. Mechanical filters, as a rule, are highly efficient. Domestic filters, even the Pasteur, are often unreliable.

In time of epidemics of cholera and typhoid even filtered water should be boiled before use, as it was found by experiments in the Medico-Chirurgical Laboratories that typhoid bacilli live longer in filtered water than in bouillon; they may even live three months.

The fewer the number of other bacteria the longer will typhoid live. They can live many days in ordinary river water.

Ice may contain great numbers of bacteria; it is well known that freezing does not destroy pathogenic bacteria, such as the typhoid bacillus. Prudden found typhoid bacilli in ice after one hundred days, although the number was greatly reduced over that placed in the ice originally. Many are squeezed out by contraction of the water. The greatest danger from ice is in dirty handling.

Disposal of sewage is a bacteriological process in many cases; either the sewage may be treated in sand filters or it may be run out on land where over 200,000 gallons may be disposed of on an acre of land a day. As far as possible nature should be imitated in every way and the breaking up of masses of matter in sewage may be accomplished in the septic-tank process in which active oxidization of the matter is accomplished by bacteria. It appears from the observations of many sanitarians that both aerobic and anaerobic bacteria are necessary to finally reduce sewage to the elementary gases and pure water.

In the interior of closed tanks and in the depths of sand filters anaerobic conditions prevail. On beds of coke, and on the surface of sand filters, aerobic conditions obtain. The effluent from a septic-tank sewage-disposal plant is very often pure water from both chemical and bacteriological standpoints, due to the chemical action of the bacteria.

Bacteriology of the Air

That the lower layers of the earth's atmosphere contain many bacteria is well known. The air over the sea and over mountain ranges is freer from bacteria than the air over arable lands and large cities.

When air is still and confined, all bacteria, according to Tyndall, gravitate to the ground, and the air above becomes quite sterile. The atmosphere of sick rooms, hospitals, public conveyances, theatres, etc., contains many bacteria and often pathogenic ones.

The pus cocci, tubercle bacilli, and the organisms causing small-pox, scarlet fever, and measles, all may contaminate the air.

The number of bacteria in a given quantity of air may be accurately measured by means of a Sedgwick-Tucker aerobioscope; this consists of a large cylindrical glass vessel opening at either end into various tubulations (Fig. 82). Into one of these granulated sugar may be packed; the ends are then plugged with cotton and the apparatus sterilized. To examine the air, a litre or more is drawn through the sugar and the latter is then shaken into the large cylinder where it is dissolved in melted gelatine culture



FIG. 82.—Sedgwick-Tucker aerobioscope. (Williams.)

media. The latter is distributed over the interior of the glass and allowed to harden. All the bacteria that were in a litre of air having been mixed with gelatine and those that are not strict anaerobes grow in the gelatine and a number of colonies can then be counted.

The dust of dwellings and streets contains most of the bacteria. Dried sputum is ground under foot and swept up in gusts of wind, and the contained bacteria are thus inhaled and do harm. The air coming quietly from the lungs is pure and sterile. Even in active disease processes of the throat this is true. In case the breath comes violently, as in speaking, coughing, and sneezing, the reverse is the case. In general it may be put down as an axiom that disease germs cannot rise from a fluid, such as sewage. If they could it would mean that they are lighter than air, which is not the case. Sewer gas, as a rule, is a bearer of some pathogenic bacteria chiefly cocci but in reality it is purer than generally supposed. The spread of organisms from sewage only extends 3–6 metres into the atmosphere and then only the the bursting of bub-

bles in the presence of gas under pressure; air currents may of course carry germs so freed a much longer distance.

Bacteriology of the Soil

At least two forms of pathogenic bacteria are habitually found in the soil. The tetanus bacillus, it is well known, exists in garden earth, manure, and top soil generally. Dirt getting into wounds is the most frequent cause of tetanus. Drinking water laden with soil has been known to have in it tetanus bacilli, and if used in an unsterilized condition in wounds or when a comparatively feeble antiseptic, such as creolin, has been added, it may cause tetanus.

The gaseous edema group, the bacilli of malignant edema, symptomatic and parasitic anthrax are frequently found in soil. The highly tilled soil of the battlefields in France was heavily laden with the first, hence the great incidence of infection after wounds in the late war. Streptococci and colon bacilli, too, have been found in garden soil. Typhoid bacilli may contaminate soil, but do not multiply in it. In sandy soil 100,000 bacteria per gram have been found, in garden soil 1,500,000 bacteria per gram, and in sewage-polluted soil 115,000,000 bacteria per gram have been determined. The first few inches of ordinary soil contain most of the bacteria, after a depth of 2 metres no bacteria at all are found and the earth is sterile.

Soil may be collected in sterile sharp-pointed iron tubes, and diluted with sterile water of given quantity and plates poured from it.

Arable lands may be enriched very much by inoculating them with certain nitrifying bacteria, some of which convert ammonia into nitrous acid, which form in them nitrites; others change nitrites into nitrates (nitrosomonas). Certain of these bacteria are concerned in the assimilation of nitrogen from the atmosphere and adding to the nitrogen content of the soil, thus enriching it. On the roots of some plants, alfalfa, beans, peas, and clover, minute tubercles develop. These little growths are caused by the

nitrifying bacteria, and add to the nutrition of the plant by adding to it ammonia.

Bacteriology of Cow's Milk

Theoretically the milk in the interior of the breasts of nursing women and the udders of cows is sterile. So soon as it leaves the nipple it becomes contaminated with bacteria, and by the time it reaches the pail, in the case of cow's milk, it is far from sterile.

Bacteria of the air, and dust from the cattle and bedding, at every movement of the cow, and by the agency of flies, find their way into milk and contaminate it. The number of bacteria that develops in the milk depends upon the number that reach it in the first place, the temperature of the air, and the length of time milk is kept at a temperature favorable for their multiplication. Two hundred and thirty-nine different varieties of bacteria have been isolated from milk at different times.

Pathogenic varieties of bacteria that have been found in cow's milk include the tubercle bacillus, *Streptococcus pyogenes*, *Staphylococcus aureus*, the colon bacillus, typhoid bacillus, the diphtheria bacillus, and a whole host of bacteria that sour or ferment the milk and render it unwholesome or poisonous for young children.

Cattle may be tuberculous, and the tubercle bacilli may reach the milk in this way. There may be abscesses of the udder and the streptococci from the pus may cause infection in those that use it. Ordinary follicular tonsillitis may be caused in this way.

Bacteria may develop rapidly in milk, which is a good culture medium, until they number many millions per cubic centimeter (sometimes 200,000,000).

In good milk the number of bacteria may increase when the temperature is 90°F., from 5,200 originally in the milk immediately after milking, to 654,000 in eight hours.

By exposing milk to a temperature of 165°F. for twenty to thirty minutes and quickly cooling (Pasteurization) most of the non-spore-bearing bacteria are destroyed, so that the number may

be reduced 99.999 percent by this process. The Pasteurization of milk has become an economic problem of great importance in large communities and is not, as it should be, sufficiently supervised. That method is best in which milk is held at 146°F. for thirty minutes. No harm is done to the nutritional value of the milk. One of the dangers of the method is that the commercial Pasteurizing machines are not always thoroughly clean and themselves contaminate the milk when discharging it after heating. More evidence is on the side of the second view. The practical importance of the controversy is that milk whether heated or not should be kept at a temperature at which bacteria will not multiply, under 60°F. Pasteurized milk is safest in time of typhoid epidemics.

Absolute cleanliness on the part of the milker, the use of sterilized gloves and clothes, the absence of flies, dust, and the immediate disposal of manure, the filtration of the milk after collection, the immediate cooling of it, the uses of sterilized milk cans and bottles, all lessen the bacterial content of milk. It then keeps better, and is a wholesomer and safer food for infants, especially in hot weather.

By drinking water containing typhoid bacilli cows cannot be sources of typhoid infection through the milk. The typhoid bacilli are not transmitted through the bodies and udders of the animals.

A bacteriologic examination of milk comprises a total count, the presence of colon bacilli, streptococci in pus cells, tubercle bacilli and special species as the case suggests. The first is done as given for water, as is the second. The discovery of streptococci is made by centrifugalizing a definite quantity and examining the sediment for chains, particularly in relation to leucocytes, the pus cells. Tubercle bacilli are found by injecting guinea pigs or by dissolving the milk in antiformin (1 part milk and 1 part 15 percent antiformin) warming and examining the sediment after centrifugalization.

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